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(71) Applicant (for all designated States except US): MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US/US]; 77 Massachusetts Avenue, Cambridge, MA 02139 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): GUARENTE, Leonard [US/US]; 93 Stanton Ave, Newton, MA 02466-3005 (US). KRIEGER, Monty [US/US]; 33 Woodbine Circle, Needham, MA 02494-2122 (US). LI, Xiaoling [CN/US]; 100 Vintage Drive, Chapel Hill, NC 27516 (US).

(74) Agent: AUER, Henry, E.; PROTEUS PATENT PRACTICE LLC, P.O. Box 1867, New Haven, CT 06508 (US).

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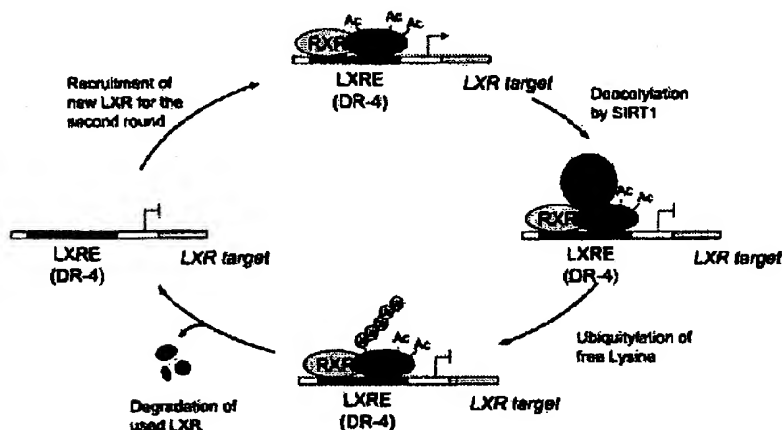
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(54) Title: CHOLESTEROL-REGULATING COMPLEX OF SIRT1 AND LXR AND METHODS OF USE



(57) Abstract: A cholesterol-regulating complex of SIRT1 and LXR and methods of use are disclosed. SIRT1 forms a complex with LXR bound to an LXR element. Methods of forming the complex, identifying an agent that modulates formation of the complex, increasing the ratio of cholesterol bound to high density lipoprotein (HDL) to total cholesterol in the plasma of a mammal, promoting ABCA1-mediated cholesterol efflux from a mammalian cell, treating a subject deemed to have a level of SIRT1 activity that is below normal, assessing whether a candidate substance modulates an LXR-dependent process, and assessing whether a candidate substance modulates an SIRT1-dependent effect of an LXR are disclosed.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Cholesterol-Regulating Complex of SIRT1 and LXR and Methods of Use

BACKGROUND OF THE INVENTION

Cholesterol homeostasis is maintained by a balance between dietary intake, *de novo* synthesis, transport, metabolism and excretion. Low levels of high density lipoprotein (HDL) and high levels of low density lipoprotein (LDL) are associated with hypercholesterolemia, hypertriglyceridemia, and increased risk of cardiovascular disease¹ and Alzheimer's disease²⁻⁶, age-associated diseases that are major causes of mortality in middle-aged and older people. Both genetic factors and the environment contribute to the progression of cardiovascular disease and Alzheimer's disease, and the risk of these disorders rises with age. However, the underlying mechanisms by which genetic factors sense the environment to mediate these ageing-associated diseases are poorly understood.

Silent information regulator 2 (Sir2) is a critical regulator of life span in response to environmental changes. The Sir2 gene is a longevity determinant in yeast, *C. elegans*⁷, and *Drosophila*^{8,9}. In both yeast^{10,11} and *Drosophila*^{8,9}, Sir2 activity is also required for lifespan extension provided by calorie restriction. Biochemically, Sir2 and its homologues (sirtuins) are a group of highly conserved NAD⁺-dependent protein deacetylases¹²⁻¹⁴. The requirement for NAD⁺ may enable sirtuins to monitor cellular metabolism and modulate cellular processes that affect ageing. In mammals, SIRT1, a mammalian orthologue of the Sir2 protein, has multiple protein substrates and is able to regulate ageing-related processes, such as cell cycle, apoptosis, oxidative stress response, and neurodegeneration¹⁵⁻²⁰. In these processes, SIRT1 is able to shift the balance between cell death and cell survival, thereby providing stress resistance²¹.

Cholesterol and fat metabolism are regulated by many common cellular and environmental factors. Peroxisome Proliferator-Activated Receptors (PPARs) and Liver X Receptors (LXRs), two subclasses of lipid-sensing nuclear receptors, play critical roles in lipid and carbohydrate metabolism. Recent studies showed that in white fat tissue, SIRT1 interacts with the nuclear receptor PPAR γ through nuclear receptor co-repressor (N-CoR) and promotes the fat mobilization upon food deprivation²². SIRT1 is also able to interact with and modify the PPAR γ coactivator PGC-1 α to regulate hepatic glucose homeostasis^{23,24}. U.

S. Patent 6,048,903 reports that the level of heavy density lipoproteins (HDL) in the blood of a human subject can be increased by administering trans-resveratrol to the subject, and that this substance reduces the level of light density lipoproteins (LDL) in the blood of the subject.

The involvement of SIRT1 in fat metabolism and glucose homeostasis affords the potential that this protein could also regulate cholesterol homeostasis in mammals. The present invention addresses these novel mechanisms of cholesterol homeostasis.

SUMMARY OF THE INVENTION

The present invention relates generally to compositions and methods useful in the field of cholesterol homeostasis and reverse cholesterol transport.

In a first aspect the invention provides an isolated complex that contains a mammalian SIRT1 protein and a mammalian LXR protein. In certain embodiments this complex additionally contains an LXR response element.

In a related aspect the invention provides a method of forming a complex that contains a mammalian SIRT1 protein and a mammalian LXR protein. The method includes combining compositions containing a mammalian SIRT1 protein, a mammalian LXR protein and a fragment of a cellular nucleic acid that includes a LXR response element.

In still a further aspect the invention provides a method of identifying an agent that modulates formation of a complex comprising a mammalian SIRT1 protein and a mammalian LXR protein. This method includes steps of

combining compositions that contain a mammalian SIRT1 protein, a mammalian LXR protein and a fragment of a cellular nucleic acid that includes a LXR response element, thus providing a complex composition;

further either contacting one of the composition containing the mammalian SIRT1 protein, the composition containing the mammalian LXR protein, or the composition containing the cellular nucleic acid fragment with a fourth composition including the agent prior to the combining step, or contacting the complex composition with the fourth composition including the agent after the combining step; and

determining whether formation of the complex is modulated by the agent.

In certain embodiments of this method the agent increases formation of the complex. In additional embodiments, the determining is conducted by comparison with a control composition not containing the agent.

In yet an additional aspect the invention provides a method of increasing the ratio of cholesterol bound to high density lipoprotein (HDL) to total cholesterol in the plasma of a mammal wherein the method includes administering to the mammal an agent that stimulates SIRT1 activity. In advantageous embodiments the agent includes T0901317.

In still a further aspect the invention provides a method of increasing the ratio of cholesterol bound to high density lipoprotein (HDL) to total cholesterol in the plasma of a mammal wherein the method includes administering to the mammal an agent that promotes formation of a complex containing a mammalian SIRT1 protein and a mammalian LXR protein. In advantageous embodiments of this method the agent includes 22(R)-hydroxycholesterol or 9-cis retinoic acid, or both.

In yet an additional aspect the invention provides a method of promoting ABCA1-mediated cholesterol efflux from a mammalian cell wherein the method includes introducing into the cell a nucleic acid that contains a sequence encoding a protein deacetylase. In several embodiments of this method the protein deacetylase is a eukaryotic Sir2 or a mammalian SIRT1. Still other embodiments of this method include a step of further contacting the cell with an agent that stimulates SIRT1 activity, such as T0901317. In other embodiments of this method the cell is further contacted with an agent that promotes formation of a complex containing a mammalian SIRT1 protein and a mammalian LXR protein. In certain embodiments the complex-promoting agent includes 22(R)-hydroxycholesterol or 9-cis retinoic acid, or both.

A further aspect of the invention provides a method of treating a subject deemed to have a level of SIRT1 activity that is below normal. Such a subject exhibits a level of HDL-cholesterol, and a ratio of HDL-cholesterol to LDL-cholesterol, that are deemed to be below normal. The method includes administering a nucleic acid encoding species-specific SIRT1 to the subject wherein the nucleic acid is effective to express a therapeutically effective amount of SIRT1 in a cell of the subject.

In a further aspect the invention provides a method for assessing whether a candidate substance modulates an LXR-dependent process. The method includes transfecting a cell with a plasmid harboring a reporter gene operably driven by an LXRE; contacting the cell with the candidate; and determining whether the candidate modulates the expression of the reporter gene in comparison with a cell not contacted with the candidate; such that a difference detected between the presence and absence of the candidate indicates that the candidate modulates the LXR-dependent process.

In an additional aspect the invention provides a method for assessing whether a candidate substance modulates an SIRT1-dependent effect of an LXR. This method includes transfecting a cell with a plasmid harboring an SIRT1 gene; further transfecting the cell with a plasmid harboring a reporter gene operably driven by an LXRE promoter; contacting the cell with the candidate; and determining whether the candidate modulates the expression of the reporter gene in comparison with a cell not contacted with the candidate; whereby a difference detected between the presence and absence of the candidate indicates that the candidate modulates the SIRT1-dependent effect of an LXR.

In a further aspect the invention provides a method for assessing whether a candidate substance modulates the interaction of SIRT1 with an LXR. This method includes transfecting a cell with a vector harboring a sequence encoding an epitope-tagged LXR; further transfecting the cell with a vector harboring a sequence encoding an SIRT1; contacting the cell with the candidate substance; lysing the cells, contacting the cell lysates with an antibody specific for the epitope tag, and recovering immunoprecipitates comprising a complex of the SIRT1 and the LXR with an antibody-specific adsorbent; carrying out a Western blot procedure using antibodies specific for SIRT1 and an LXR; whereby a difference in complex formation detected in the presence of the candidate compared with the absence of the candidate indicates that the candidate modulates the interaction of the SIRT1 with the LXR.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Loss of function of SIRT1 results in altered cholesterol homeostasis.

(a-c) Plasma samples from wildtype (filled bar), *SIRT1*^{+/-} (bar with slanted shading), and *SIRT1*^{-/-} (open bar) mice were analyzed for (a) total cholesterol, (b) HDL cholesterol, (c) LDL cholesterol.

(d) FPLC plasma lipoprotein cholesterol profiles in wildtype (filled diamond) and *SIRT1*^{-/-} (open diamond) mice.

(e) Relative lipid contents in HDL fractions of wildtype (filled bar) and *SIRT1*^{-/-} (open bar) mice.

- (f) Total cholesterol from wildtype control (filled bar) and littermate *SIRT1*^{-/-} (open bar) testes and livers.
- (g) ApoAI-mediated efflux of [³H]cholesterol from primary mouse embryonic fibroblasts (MEFs) (left panel) or THP-1 human monocytes (right panel), with or without pretreatment with nicotinamide.

Figure 2. Expression of ABCA1 and interaction with LXR nuclear receptors.

- (a) Expression of ABCA1 mRNA in livers (left panel, determined by quantitative real-time PCR), and in testes and ovaries (right panel, determined by northern blots of wildtype (+/+) and *SIRT1*^{-/-} (-/-) animals).
- (b) Inhibition of SIRT1 activity reduces ABCA1 promoter activity.
- (c) Effect of SIRT1 deficiency on ABCA1 promoter activity in MEFs.
- (d) Interaction of SIRT1 with the LXRE of ABCA1 promoter.
- (e) SIRT1 interacts with LXR α and LXR β .

Figure 3. SIRT1 deficiency compromises the normal responses to a LXR agonist *in vivo*.

- (a) LXR target mRNAs in livers of *SIRT1*^{-/-} mice.
- (b) Relative levels of LXR target mRNAs in livers of mice treated with the LXR agonist T0901317.
- (c) ABCA1 protein levels in wildtype and *SIRT1*^{-/-} mice without or with T0901317.
- (d) Triglyceride levels after T0901317 administration. in *SIRT1*^{-/-} mice accumulate lower levels of triglycerides in plasma and liver.
- (e) Induction of cholesterol in plasma after T0901317 feeding.
- (f) Representative FPLC plasma lipoprotein profiles in wildtype and *SIRT1*^{-/-} mice before (filled square) and after (open square) T0901317 feeding.

Figure 4. SIRT1 regulates LXR stability and activity by deacetylating the LXR protein.

- (a) Effect of SIRT1 on LXR Protein Expression *SIRT1*.

- (b) Effect of knockdown of SIRT1 by RNA interference on the level of LXR.
- (c) Effect of inhibitors on protein expression of LXR
- (d) SIRT1 promotes the ubiquitination of LXR.
- (e) Acetylation of LXR *in vivo* and activation of LXR by 22(R)-HC and 9-cisRA.
- (f) SIRT1 deacetylates HA-LXR β .

Figure 5. Schematic representation of the effect of inhibition of SIRT1 or proteasome-mediated LXR degradation on the transcription activity of LXR on the ABCA1 promoter.

DETAILED DESCRIPTION OF THE INVENTION

Table of Sequences

Table 1. Table of Sequences in the Disclosure

SEQ ID NO:	IDENTIFICATION	GENBANK ACC. NO.	TYPE
1	Human SIRT1	NP_036370	Prot
2	Murine SIRT1	NP_062786	Prot
3	Human Liver X Receptor alpha	NP_062786	Prot
4	Human Liver X Receptor beta	NP_009052.3	Prot
5	Murine Liver X Receptor alpha.	NP_038867.1	Prot
6	Murine Liver X Receptor beta	NP_033499.1	Prot
7	Human SIRT1	NM_012238	mRNA
8	Murine SIRT1	NM_019812	mRNA
9	Human liver X receptor alpha	NM_005693	mRNA
10	Human liver X receptor beta	NM_007121	mRNA
11	Murine liver X receptor alpha	NM_013839	mRNA
12	Murine liver X receptor beta	NM_009473	mRNA
13	Primer		DNA
14	Primer		DNA
15	Primer		DNA
16	Primer		DNA
17	Primer		DNA
18	Primer		DNA
19	Primer		DNA
20	Primer		DNA

As used herein, HDL (high-density lipoprotein) relates to a class of plasma lipoprotein with a high proportion of protein, including apolipoproteins A, C, D and E. HDL incorporates and transports cholesterol, whether free or esterified, in the plasma as an HDL-

cholesterol complex. The term HDL may be used in a context-dependent manner to designate cholesterol bound to HDL particles.

As used herein, LDL (low-density lipoprotein) relates to a class of plasma lipoprotein with a high proportion of lipid, including cholesterol, cholesterol esters and triglycerides. It includes primarily apolipoprotein B-100 and apolipoprotein E. LDL incorporates and transports cholesterol in the plasma. The term LDL may be used in a context-dependent manner to designate cholesterol bound to LDL particles.

As used herein, the term "isolated" and similar terms relate to a substance that has been purified from a naturally occurring state by the removal of at least one component with which the substance occurs in the natural state. The term "purified" may be used synonymously with "isolated". It is understood that these terms do not require that the substance have any predetermined level of purity.

As used herein, the term "a complex" and similar terms relate to a combination of two or more substances combined with each other sufficiently stably to undergo an isolation procedure and be characterized as being so combined. The components of the complex may be bound directly to each other, or the combination may include an intermediary facilitating component such that the identified components are not directly in contact with one another. A complex may be formed by noncovalent interactions binding the components or by covalent bonds between them.

As used herein the term "cholesterol" generally refers to total occurrence of cholesterol as both cholesterol and cholesteryl esters. In certain contexts which will be apparent to the worker of skill in the art, "cholesterol" may be used herein to refer only to free cholesterol.

As used herein, the term "treatment" and similar terms and phrases relate to the application or administration of a therapeutic agent to a subject having a disease or condition, a symptom of disease, or a predisposition toward a disease, or application or administration of a therapeutic agent to an isolated tissue or cell line from the subject. Treatment is intended to promote curing or healing thereof, or to alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptoms of disease, or the predisposition toward disease.

As used herein, the phrases "therapeutically effective amount" and "prophylactically effective amount" refer to an amount that provides a therapeutic benefit in the treatment of a disease, or an effect providing prevention or diminishing the severity of the disease, respectively. The specific amount that is therapeutically effective can be readily determined

by an ordinary medical practitioner employing assessment of response in a treated subject, and may vary depending on factors known in the art, such as the nature of the disease, the subject's history and age, the stage of disease, and the administration of other therapeutic agents.

As used herein, a "pharmaceutical composition" relates to a composition that includes a pharmacologically effective amount of a polynucleotide and a pharmaceutically acceptable carrier. As used herein, "pharmacologically effective amount," "therapeutically effective amount" or simply "effective amount" refers to that amount of an inhibitory polynucleotide effective to produce the intended pharmacological, therapeutic or preventive result. For example, if a given clinical treatment is considered effective when there is at least a minimal measurable change in a clinical parameter associated with a disease or disorder, a therapeutically effective amount of a drug for the treatment of that disease or disorder is the amount necessary to effect at least extent of change in the parameter.

The term "pharmaceutically acceptable carrier" refers to a composition for administration of a therapeutic agent that is at least both physiologically acceptable and approvable by a regulatory agency.

SIRT1 Polypeptides

As used herein, the terms an "SIRT1 polypeptide", an "SIRT1 protein", and related terms and phrases, relate to wild type SIRT1, to a mutant SIRT1, a variant SIRT1, and to biologically active fragments and mature forms thereof. An important SIRT1 protein to be used in the present invention is human SIRT1. The amino acid sequence of human SIRT1 is given in GenBank Acc. No. NP_036370, disclosed as being composed of 747 amino acid residues, is shown in Table 2 using the conventional one-letter amino acid code (International Union Of Biochemistry And Molecular Biology, Recommendations on Biochemical & Organic Nomenclature, Symbols & Terminology etc., Part 1, Section A: Amino-Acid Nomenclature, Section 3AA-1. Names Of Common Alpha-Amino Acids, <http://www.chem.qmul.ac.uk/iubmb/> and *J. Biol. Chem.*, 1985, 260,14-42).

Table 2. Amino Acid Sequence of Human SIRT1.

```

1 madeaalalq pggspsaaga dreasspag eplrkprprd gpglerspge ppgaaperev
61 paaargcpga aaaalwreae aeaaaaggeq eaqataaage gdngpglqgp sreppladnl
121 ydeddddege eeeeeaaaai gyrdnllfgd eiitngfhsc esdeedrash assdwtprp
181 rigpytfvqq hlmigtdprt ilkdllpeti pppelddmtl wqiviniise pprkkrkdi
241 ntiedavkll qeckkiivlt gagvsvscgi pdfrrsrdgiy arlavdfpdl pdpqamfdie
301 yfrkdprpff kfakeiypgq fqpslchkfi alsdkegkll rnytqnidtl eqvagiqrrii

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361 qchgsfatas clickykvd eavrgdifnq vvprcprcpa deplaimkpe ivffgenlpe
421 qfhramkydk devdllivig sslkvrpval ipssiphevp qilinreplp hlhfdvellg
481 dcdviinelc hrlggeyakl ccnpvklsei tekpprtqke laylselppt plhvsedsss
541 pertsppdss vivtllldqaa ksnddlldvse skgcmeekpq evqtsrnves iaegmenpdl
601 knvgsstgek nertsvagtv rkcpwnrvak eqisrrldgn qylflppnry ifhgaevysd
661 seddvlssss cgsnsdsgtc qspsleepme deseieefyn gledepdvep raggagfgtd
721 gddqeainea isvkqevtdm nypsnks

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(SEQ ID NO:1)

The amino acid sequence of murine SIRT1 is given in GenBank Acc. No. NP_062786, disclosed as being composed of 737 amino acid residues, is shown in Table 3.

Table 3. Amino Acid Sequence of Murine SIRT1.

```

1 madevalalq aagspaaaaa meaasqpade plrkrprrdg pglgrspgep saavapaaaag
61 ceaasaaapa alwreaagaa asaereapat avagdgdnsg glrrepraad dfdddegeee
121 deaaaaaaaaa aigyrnlll tdglltngfh scesddddrt shasssdwtp rprigpytfv
181 qqhlmigtdp rtilkdllpe tippelddm tlwqivini seppkrkrk dintiedavk
241 llqeckkiiv ltgagvspsc gipdfrrsdg iyarlavdfr dlpdpqamfd ieyfrkdprp
301 ffkfakeiyp gqfqpslchk fialsdkegk llrnytqnid tleqvagiqr ilqchgsfat
361 asclickykv dceavrgdif nqvprcprc padeplaimk peivffgenl peqfhamky
421 dkdevdliiv igsslkvrpv alipssiphe vpqilinrep lphlhfdvel lgdcdviine
481 lchrlggeya klccnpvkl eitekpprpq kelvhlself ptplhiseds sspertvpqd
541 ssviatlvdq atnnvnndle vsesscveek pqevqtsrnv eninvenpdf kavgsstadk
601 nertsvaetv rkcpwnrlak eqiskrlegn qylfvppnry ifhgaevysd seddvlssss
661 cgsnsdsgtc qspsleeple deseieefyn gledtderpe caggsgfgad ggdqevvnea
721 iatrqeltdv nypsdks

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(SEQ ID NO:2)

The amino acid sequence of human liver X receptor alpha is given in GenBank Acc. No. NP_062786, disclosed as being composed of 447 amino acid residues, is shown in Table 4.

Table 4. Amino Acid Sequence of Human Liver X Receptor alpha.

```

MSLWLGA VPDI PPDS AVELWKPGAQDASSQAQGGSSC ILREEA
RMPHSAGGTAGVGLEAAEPTALLTRAEPPEPTEIRPQKRKKGPAPKMLGNELCSVCG
DKASGFHYNVLSCEGCKGFFRRSVIKGAHYICHSGGHCPMDTYMRRKCQECRLRKCRQ
AGMREECVLSEEQIRLKKLKRQEEQAHATSLPPRRSSPPQILPQLSPEQLGMIEKLV
AAQQQCNRRSFSDRLRVTPWPMAPDPHSREARQORFAHFTELAI VSVQEI VDFAKQLP
GFLQLSREDQIAL LKTS AIEV MLLET SRRYNPGSESITFLKDFSYNREDFAKAGLQVE
FINPIFEFSRAMNELQLNDAEFALLIAISIFSADRPNVQDQLQVERLQHTYVEALHAY
VSIHHPH DRLMFPRMLMKLVSLRTLSSVHSEQVFALRLQDKKLPLLSEIWDVHE

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(SEQ ID NO:3)

The amino acid sequence of human liver X receptor beta is given in GenBank Acc. No. NP_009052.3, disclosed as being composed of 461 amino acid residues, is shown in Table 5.

Table 5. Amino Acid Sequence of Human Liver X Receptor beta.

MSSPTTSSLDTPLPNGPPQPGAPSSSPTVKEEGPEPWPGGPDP
 DVPGTDEASSACSTDWVIPDPEEEPERKRKKGPAPKMLGHELRCRVCGDKASGFHYNVL
 SCEGCKGFFRRSVVRGGARRYACRGGGTCQMDAFMRRKCQQCRLRKCKEAGMREQC
 VLSCEEQIRKKKIRKQQQQESQSQSQSPVGPQGSSSSASGPGASPGGSEAGSQGS
 GEGEVQLTAAQELMIQQLVAAQLQCNKRSFSDQPKVTPWPLGADPQSRDARQORFAH
 FTELAIISVQEIIVDFAKQVPGFLQLGREDQIALLKASTIEIMLLETARRYNHETECIT
 FLKDFTYSKDDFHRAGLQVEFINPIFEFSRAMRRLGLDDAEYALLIAINIFSADRP
 NVQEPGRVEALQOPYVEALLSYTRIKRPQDQLRFPRMLMKLVSLRTLSSVHSEQV
 FALRLQDKKLPLLSEIWDVHE

(SEQ ID NO:4)

The amino acid sequence of murine liver X receptor alpha is given in GenBank Acc. No., NP_038867.1 disclosed as being composed of 445 amino acid residues, is shown in Table 6.

Table 6. Amino Acid Sequence of Murine Liver X Receptor alpha.

MSLWLEASMPDVSPDSATELWKTEPQDAGDQGGNTCILREARM
 POSTGVALGIGLESAEPTALLPRAETLPEPTELRPQKRKKGPAPKMLGNELCSVCGDK
 ASGFHYNVLSCEGCKGFFRRSVIKGARYVCHSGGHCPMDTYMRRKCQECRLRKCRQAG
 MREECVLSEEQIRLKKLKRQEEEAQATSVS PRVSSPPQVLPQLSPEQLGMIEKLVA
 AQQCNRRSFSDRLRVTPWPIAPDPQSRREARQORFAHFTLAIISVQEIIVDFAKQLPGF
 LQLSREDQIALLKTS AIEVMLLETSSRYNPGSESITFLKDFSYNREDFAKAGLQVEFI
 NPIFEFSRAMNELQLNDAEFALLIAISIFSADRPNVQDQLQVERLQHTYVEALHAYVS
 INHPHDLRMFPRMLMKLVSLRTLSSVHSEQV FALRLQDKKLPLLSEIWDVHE

(SEQ ID NO:5)

The amino acid sequence of murine liver X receptor beta is given in GenBank Acc. No. NP_033499.1, disclosed as being composed of 446 amino acid residues, is shown in Table 7.

Table 7. Amino Acid Sequence of Murine Liver X Receptor beta.

MSSPTSSLDTPVPGNGSPQPSTSATSPITKEEGQETDPPPGSEG
 SSSAYIVVILEPEDEPERKRKKGPAPKMLGHELRCRVCGDKASGFHYNVLSCEGCKGFF
 RRSVVHGGAGRYACRSGTCQMDAFMRRKCQLCRLRKCKEAGMREQC
 VLSCEEQIRKKRIQKQQQQPPPPSEPAASSSGRPAASPGTSEASSQGS
 GEGEGIQLTAAQELMIQQLVAAQLQCNKRSFSDQPKVTPWPLGADPQSRDARQORFAH
 FTELAIISVQEIIVDFAKQVPGFLQLGREDQIALLKASTIEIMLLETARRYNHETECIT
 FLKDFTYSKDDFHRAGLQVEFINPIFEFSRAMRRLGLDDAEYALLIAINIFSADRP
 NVQEPSRVEALQOPYVEALLSYTRIKRPQDQLRFPRMLMKLVSLRTLSSVHSEQV
 FALRLQDKKLPLLSEIWDVHE

(SEQ ID NO:6)

In general, an "SIRT1 polypeptide" and various "LXR polypeptides" as employed in the methods and compositions of the present invention, includes wild type human SIRT1 and LXR polypeptides or wild type murine SIRT1 and murine LXR polypeptides such as shown

in Tables 2-7, as well as wild type vertebrate orthologs thereof, and domains, motifs and fragments thereof. In addition, an these terms include recombinant mutant polypeptides, domains, motifs and fragments in which at least one amino acid residue has been changed to a different amino acid residue; or one or more residues may be deleted; or one or more residues may be inserted between neighboring residues in an original sequence. A mutant or variant SIRT1 polypeptide may have from 1 amino acid residue up to 1% of the residues changed, or up to 2%, or up to 5%, or up to 8%, or up to 10%, or up to 15%, or up to 20%, or somewhat higher percent, of the residues changed from a wild type or reference sequence. The recombinant mutant or variant polypeptides, domains, motifs and fragments of SIRT1 are used in the present methods and compositions as long as they demonstrably exhibit at least one biological activity or function of wild type SIRT1 and LXRs. Possession of a biological activity or function may be determined by a worker of skill in the fields related to the present invention, including, by way of nonlimiting example, molecular biology, cell biology, pathology, clinical medicine and the like. Such workers of skill in the fields of the invention may assay recombinant mutant SIRT1 and LXR polypeptides, domains, motifs and fragments at least by methods described in the Examples of the present invention.

It will be recognized in the art that an amino acid sequence of an SIRT1 or LXR polypeptide can be varied without significant effect on the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be certain areas on the protein that are important for its activity. In general, it is possible to replace residues that form the tertiary structure, provided that residues providing a similar function are used. In other instances, the type of residue may be completely unimportant if the alteration occurs at a non-important region of the protein.

Nucleic Acids

As used herein, the term "SIRT1 polynucleotide" or "SIRT1 nucleic acid", or "LXR polynucleotide" or "LXR nucleic acid" or related terms and phrases, relates to any polynucleotide that encodes any SIRT1 polypeptide as described herein. In general, any nucleotide sequence that encodes an SIRT1 polypeptide described above is encompassed within the present invention. In some embodiments, a nucleic acid encoding a polypeptide having the amino acid sequence of a human SIRT1 shown in Table 2 includes a coding sequence of the mRNA nucleic acid sequence disclosed in GenBank Acc. No. NM_012238,

shown in Table 8, or a fragment thereof. In Table 8, the coding sequence extends from position 54 to position 2297.

Table 8.

1	gtc	gag	cgg	g	agc	agag	gag	g	c	gag	ggg	agg	g	ccg	agg	g	aat	g	gg	c
61	acg	agg	c	ggc	c	tc	g	c	c	c	c	c	g	g	c	c	c	c	c	c
121	agg	c	gc	gc	gt	c	c	c	c	c	g	g	c	c	c	c	c	c	c	c
181	gc	ct	c	gag	c	g	g	c	c	c	g	g	c	c	c	c	c	c	c	c
241	c	gg	c	cag	g	g	g	c	c	g	g	c	c	c	c	c	c	c	c	c
301	c	gg	c	g	g	c	g	g	c	g	g	c	c	c	c	c	c	c	c	c
361	at	g	g	g	c	c	g	g	c	c	c	c	c	c	c	c	c	c	c	c
421	a	ag	a	c	g	a	c	g	a	g	g	c	c	c	c	c	c	c	c	c
481	g	a	g	a	t	a	c	c	t	t	c	g	g	t	c	c	c	c	c	c
541	a	t	g	a	g	a	g	a	g	a	g	a	g	a	g	a	g	a	g	a
601	g	t	c	c	a	t	a	t	a	c	c	c	c	c	c	c	c	c	c	c
661	a	a	g	a	t	t	t	a	t	t	c	c	c	c	c	c	c	c	c	c
721	t	t	g	t	t	a	t	t	a	t	a	a	a	a	a	a	a	a	a	a
781	t	t	g	a	a	t	a	t	a	t	a	a	a	a	a	a	a	a	a	a
841	g	g	g	t	g	t	g	t	g	t	g	t	g	t	g	t	g	t	g	t
901	t	t	g	c	t	g	t	a	g	a	c	c	a	a	a	a	a	a	a	a
961	g	a	a	a	a	a	t	c	c	c	c	c	c	c	c	c	c	c	c	c
1021	c	a	t	c	t	c	t	c	t	c	c	c	c	c	c	c	c	c	c	c
1081	a	t	a	c	c	c	a	a	a	c	a	a	a	a	a	a	a	a	a	a
1141	a	t	g	g	t	t	c	c	c	t	t	c	c	c	c	c	c	c	c	c
1201	t	a	c	g	a	g	a	g	a	g	a	g	a	g	a	g	a	g	a	g
1261	c	g	c	t	t	g	c	t	a	t	c	a	a	a	a	a	a	a	a	a
1321	a	t	a	g	a	g	c	c	a	t	a	a	a	a	a	a	a	a	a	a
1381	t	c	a	a	a	g	t	a	a	g	t	a	a	a	a	a	a	a	a	a
1441	t	a	a	t	a	a	t	a	t	a	a	a	a	a	a	a	a	a	a	a
1501	a	t	g	t	c	a	a	a	t	a	a	a	a	a	a	a	a	a	a	a
1561	a	c	c	c	t	g	t	a	a	a	a	a	a	a	a	a	a	a	a	a
1621	a	t	t	t	g	t	c	a	g	a	a	a	a	a	a	a	a	a	a	a
1681	g	a	a	c	t	t	c	a	c	c	c	c	c	c	c	c	c	c	c	c
1741	a	t	g	a	t	g	a	t	t	g	a	t	t	g	a	t	t	g	a	t
1801	a	a	a	c	t	t	c	t	a	g	a	a	a	a	a	a	a	a	a	a
1861	t	t	g	g	t	t	c	t	a	g	a	a	a	a	a	a	a	a	a	a
1921	g	c	t	g	g	c	c	t	a	a	a	a	a	a	a	a	a	a	a	a
1981	t	g	t	t	t	t	g	c	c	a	a	a	a	a	a	a	a	a	a	a
2041	a	t	g	a	c	g	t	c	t	t	a	t	a	t	a	t	a	t	a	t
2101	c	a	a	g	t	t	t	a	g	a	a	a	a	a	a	a	a	a	a	a
2161	a	a	g	a	t	g	a	g	c	c	c	c	c	c	c	c	c	c	c	c
2221	a	t	c	a	a	a	g	a	g	c	c	c	c	c	c	c	c	c	c	c
2281	c	a	t	c	a	a	a	c	a	a	a	a	a	a	a	a	a	a	a	a
2341	g	a	a	c	t	t	t	a	g	c	a	a	a	a	a	a	a	a	a	a
2401	a	a	g	g	t	g	t	a	a	t	a	a	a	a	a	a	a	a	a	a
2461	a	t	t	a	t	t	t	c	t	g	t	a	a	a	a	a	a	a	a	a
2521	a	c	t	a	a	g	t	a	t	c	a	a	a	a	a	a	a	a	a	a
2581	t	g	a	t	a	a	a	t	t	c	a	a	a	a	a	a	a	a	a	a
2641	t	t	a	a	a	g	t	t	t	t	c	a	a	a	a	a	a	a	a	a
2701	c	c	a	a	a	a	a	t	g	a	t	t	t	t	c	a	a	a	a	a
2761	t	g	t	t	a	c	g	t	a	a	c	t	t	t	t	g	a	t	a	a
2821	a	c	t	g	t	g	g	c	a	a	c	t	t	t	t	t	t	t	t	t
2881	a	c	a	a	t	a	t	g	t	g	g	a	g	a	c	a	c	a	c	a

```

2941 aatttcagga ttattgtatt tacgttcaaa tgaagatggc ttttgtactt cctgtggaca
3001 tgtagtaatg tctatattgg ctcataaaac taacctgaaa aacaaataaa tgctttggaa
3061 atgtttcagt tgcttttagaa acattagtgc ctgcctggat ccccttagtt ttgaaatatt
3121 tgccattgtt gtttaaatac ctatcactgt ggtagagctt gcattgatct tttccacaag
3181 tattaaactg ccaaaatgtg aatatgcaaa gcctttctga atctataata atggtacttc
3241 tactggggag agtgtaatat tttggactgc tgttttccat taatgaggag agcaacaggc
3301 ccctgattat acagttccaa agtaataaga tgtaattgt aattcagcca gaaagtacat
3361 gtctcccat ggaggattt ggtgttaaat accaaactgc tagccctagt attatggaga
3421 tgaacatgat gatgtaactt gtaatagcag aatagttaat gaatgaaact agttcttata
3481 atttatcttt atttaaaagc ttagcctgcc ttaaaactag agatcaactt tctcagctgc
3541 aaaagcttct agtctttcaa gaagttcata ctttatgaaa ttgcacagta agcattttatt
3601 tttcagacca tttttgaaca tcactcctaa attaataaag tattcctctg ttgctttagt
3661 atttattaca ataaaaaggg tttgaaatat agctgttctt tatgcataaa acaccagct
3721 aggaccatta ctgccagaga aaaaaatcgt attgaatggc catttcccta cttataagat
3781 gtctcaatct gaattttattt ggctacacta aagaatgcag tataatttagt tttccatttg
3841 catgatgttt gtgtgctata gatgatattt taaattgaaa agtttgtttt aaattatttt
3901 tacagtgaag actgttttca gctcttttta tattgtacat agtcttttat gtaatttact
3961 ggcatatggt ttgtagactg tttaatgact ggatatcttc cttcaacttt tgaaatacaa
4021 aaccagtgtt ttttacttgt acactgtttt aaagtctatt aaaattgtca tttgactttt
4081 ttctgtttaa aaaaaaaaaa aaaaaaa

```

(SEQ ID NO:7)

In some embodiments, a nucleic acid encoding a polypeptide having the amino acid sequence of a murine SIRT1 shown in Table 3 includes a coding sequence of the mRNA nucleic acid sequence disclosed in GenBank Acc. No. NM_019812, shown in Table 9, or a fragment thereof. In Table 9, the coding sequence extends from position 48 to position 2261.

Table 9.

```

1 gcggagcaga ggaggcgagg gcggagggcc agagaggcag ttggaagatg gcggacgagg
61 tggcgctcgc ccttcaggcc gccggctccc cttccgcggc ggccgccatg gaggccgcgt
121 cgcagccggc ggacgagccg ctccgcgaaga ggccccgccg agacgggcct ggccctcgggc
181 gcagcccggg cgagccgagc gcagcagtgg cgccggcggc cgcggggtgt gaggcggcga
241 gcgccgcggc cccggcgggc ctgtggcggg aggcggcagg ggcggcggcg agcgcggagc
301 gggaggcccc ggcgacggcc gtggccgggg acggagacaa tgggtccggc ctgcggcggg
361 agccgagggc ggctgacgac ttcgacgacg acgagggcga ggaggaggac gaggcggcgg
421 cggcagcggc ggcggcagcg atcggctacc gagacaacct cctgttgacc gatggactcc
481 tctaataatg ctttcattcc tgtgaaagtg atgacgatga cagaacgtca cacgccagct
541 ctagtgactg gactccgcgg ccgcggatag gtccatatac tttgtttcag caacatctca
601 tgattggcac cgatccctcg acaattctta aagatttatt accagaaaca attcctccac
661 ctgagctgga tgatatgacg ctgtggcaga ttgttattaa taccctttca gaaccacaa
721 agcggaaaaa aagaaaagat atcaatacaa ttgaagatgc tgtgaagtta ctgcaggagt
781 gtaaaaagat aatagttctg actggagctg ggggtttctgt ctctgtggg attctgact
841 tcagatcaag agacgtatc tatgctgcc ttgcggtgga cttcccagac ctcccagacc
901 ctcaagccat gtttgatatt gagtatttta gaaaagaccc aagaccattc ttcaagtttg
961 caaaggaaat atatcccgga cagttccagc cgtctctgtg tcacaaattc atagctttgt
1021 cagataagga aggaaaacta cttcgaaatt atactcaaaa tatagatacc ttggagcagg
1081 ttgcaggaat ccaaaggatc cttcagtgtc atgggttcct tgcaacagca tcttgccctga
1141 tttgtaataa caaagttgat tgtgaagctg ttcgtggaga catttttaat caggtagttc
1201 ctcggtgccc taggtgcccc gctgatgagc cacttgccat catgaagcca gagattgtct
1261 tctttggtga aaacttacca gaacagtttc atagagccat gaagtatgac aaagatgaag
1321 ttgacctcct cattgttatt ggatctcttc tgaaagttag accagtagca ctaattccaa

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1381 gttctataacc ccatgaagtg cctcaaatat taataaatag ggaacctttg cctcatctac
1441 attttgatgt agagctcctt ggagactgcg atgttataat taatgagttg tgtcataggg
1501 taggtggtga atatgccaaa ctttggttga accctgtaaa gctttcagaa attactgaaa
1561 aacctccacg cccacaaaag gaattgggtc atttatcaga gttgccacca acacctcttc
1621 atatttcgga agactcaagt tcacctgaaa gaactgtacc acaagactct tctgtgattg
1681 ctacacttgt agaccaagca acaaacaaca atgttaatga tttagaagta tctgaatcaa
1741 gttgtgtgga agaaaaacca caagaagtac agactagtag gaatgttgag aacattaatg
1801 tggaaaatcc agattttaag gctgttggtt ccagtactgc agacaaaaat gaaagaactt
1861 cagttgcaga aacagtgaga aaatgctggc ctaatagact tgcaaaggag cagattagta
1921 agcggcttga gggtaatcaa tacctgtttg taccaccaa tcgttacata ttccacggtg
1981 ctgaggtata ctcagactct gaagatgacg tcttgctctc tagttcctgt ggcagtaaca
2041 gtgacagtgg cacatgccag agtccaagtt tagaagaacc cttggaagat gaaagtgaaa
2101 ttgaagaatt ctacaatggc ttggaagatg atacggagag gcccgaatgt gctggaggat
2161 ctggatttgg agctgatgga ggggatcaag aggttgtaa tgaagctata gctacaagac
2221 aggaattgac agatgtaaac tatccatcag acaaatcata acactattga agctgtccgg
2281 attcaggaat tgctccacca gcattgggaa ctttagcatg tcaaaaaaat gaatgtttac
2341 ttgtgaactt gaacaaggaa atctgaaaga tgtattatgt atagactgga aaatagattg
2401 tcttcttggg taatttctaa agttccatca tttctgtttg tacttgtaaa ttcaacactg
2461 ttggttgact tcatcttcct ttcaagggtc atttgatga tacattcgta tgtatgtata
2521 attttgtttt ttgcctaatt agtttcaacc ttttaaagtt ttcaaaagcc attggaatgt
2581 taatgtaaag ggaacagctt atctagacca aagaatggta tttcacactt ttttgtttgt
2641 aacattgaat agttttaaag cctcaatttc tgttctgctg aacttttatt tttaggacag
2701 ttaacttttt aaacactggc attttccaaa acttggtggc gctaactttt taaaatcaca
2761 gatgacttgt aatgtgagga gtcagcaccg tgtctggagc actcaaaact tgggctcagt
2821 gtgtgaagcg tacttactgc atcgtttttg tacttgctgc agacgtggta atgtccaaac
2881 aggccctga gactaatctg ataaatgatt tggaaatgtg tttcagttgt tctagaaaca
2941 atagtgcctg tctatatagg tccccttagt ttgaatatgt gccattgttt aattaaatac
3001 ctatcactgt ggtagagcct gcatagatct tcaccacaaa tactgccaa atgtgaatat
3061 gcaaagcctt tctgaatcta ataatggtac ttctactggg gagagtgtaa tattttggac
3121 tgctgttttt ccattaatga ggaaagcaat aggcctctta attaaagtcc caaagtcata
3181 agataaattg tagctcaacc agaaagtaca ctggtgcctg ttgaggattt ggtgtaatgt
3241 atcccaaggt gttagccttg tattatggag atgaatacag atccaatagt caaatgaaac
3301 tagttcttag ttatttataa gcttagcttg ccttaaaact agggatcaat tttctcaact
3361 gcagaaactt ttagcctttc aaacagttca cacctcagaa agtcagtatt tattttacag
3421 acttcttttg aacattgccc ccaaatttaa atattcatgt gggtttagta tttattacaa
3481 aaaaatgatt tgaaatatag ctgttcttta tgcataaaat acccagttag gaccattact
3541 gccagaggag aaaagtatta agtagctcat ttccctacct aaaagataac tgaatttatt
3601 tggctacact aaagaatgca gtatatttag ttttccattt gcatgatgtg tttgtgctat
3661 agacaatatt ttaaattgaa aaatttggtt taaattatgt ttacagtga gactgttttc
3721 agctcttttt atattgtaca tagactttta tgtaatctgg catatgtttt gtagaccgtt
3781 taatgactgg attatcttcc tccaactttt gaaatacaaa aacagtgttt tatactaaaa
3841 aaaaaaaaaa

```

(SEQ ID NO:8)

In some embodiments, a nucleic acid encoding a polypeptide having the amino acid sequence of a human liver X receptor alpha shown in Table 4 includes a coding sequence of the mRNA nucleic acid sequence disclosed in GenBank Acc. No. NM_005693, or a fragment thereof. In Table 10, the coding sequence extends from position 36 to position 1379.

Table 10. Polynucleotide sequence encoding human liver X receptor alpha

```

1 cagtgccttg gtaatgacca gggctccaga aagagatgtc cttgtggctg ggggccctg
61 tgcctgacat tctcctgac tctgcggtgg agctgtggaa gccaggcgca caggatgcaa

```



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121 gcagccaggc ccagggaggc agcagctgca tcctcagaga ggaagccagg atgccccact
181 ctgctggggg tactgcaggg gtggggctgg aggctgcaga gcccacagcc ctgctcacca
241 gggcagagcc cccttcagaa cccacagaga tccgtccaca aaagcggaaa aaggggcccag
301 cccccaaaat gctggggaac gagctatgca gcgtgtgtgg ggacaaggcc tcgggcttcc
361 actacaatgt tctgagctgc gagggctgca agggattctt ccgccgcagc gtcatacaagg
421 gagcgcacta catctgccac agtggcggcc actgccccat ggacacctac atgctgcgca
481 agtgcagga gtgtcggctt cgcaaatgcc gtcaggctgg catgcgggag gagtgtgtcc
541 tgtcagaaga acagatccgc ctgaagaaac tgaagcggca agaggaggaa caggctcatg
601 ccacatcctt gccccccagg cgttcctcac cccccaaat cctgccccag ctgagcccgg
661 aacaactggg catgatcgag aagctcgtcg ctgccagca acagtgtaac cggcgctcct
721 tttctgaccg gcttcgagtc acgccttggc ccatggcacc agatccccat agccgggagg
781 cccgtcagca gcgctttgcc cacttctactg agctggccat cgtctctgtg caggagatag
841 ttgactttgc taaacagcta cccggcttcc tgcagctcag ccgggaggac cagattgccc
901 tgctgaagac ctctgcgata gaggtgatgc ttctggagac atctcggagg tacaaccctg
961 ggagtgaag tatcaccttc ctcaaggatt tcagttataa ccgggaagac tttgccaaag
1021 cagggtgca agtgggaattc atcaacccca tcttcgagtt ctccagggcc atgaatgagc
1081 tgcaactcaa tgatgccgag tttgccttgc tcattgctat cagcatcttc tctgcagacc
1141 ggcccaacgt gcaggaccag ctccagggtg agaggctgca gcacacatat gtggaagccc
1201 tgcattgcta cgtctccatc caccatcccc atgaccgact gatgttccca cggatgctaa
1261 tgaaactggg gagcctccgg accctgagca gcgtccactc agagcaagtg tttgactgc
1321 gtctgcagga caaaaagctc ccaccgctgc tctctgagat ctgggatgtg cacgaatgac
1381 tgttctgtcc ccatattttc tgttttcttg gccggatggc tgaggcctgg tggtgcctc
1441 ctagaagtgg aacagactga gaagggcaa cattcctggg agctgggcaa ggagatcctc
1501 ccgtggcatt aaaagagagt caaagggt

```

(SEQ ID NO:9)

In some embodiments, a nucleic acid encoding a polypeptide having the amino acid sequence of a human liver X receptor beta shown in Table 5 includes a coding sequence of the mRNA nucleic acid sequence disclosed in GenBank Acc. No. NM_007121, or a fragment thereof.

In Table 11, the coding sequence extends from position 259 to position 1644.

Table 11. Polynucleotide sequence encoding human liver X receptor beta

```

1 ctcttccgga cgtgacgcaa gggcgggggt gccggaagaa gtggcgaagt tactttttag
61 ggtattttgag tagcggcggt gtgtcagggg ctaaagagga ggacgaagaa aagcagagca
121 agggaaacca gggcaacagg agtagttcac tccgcgagag gccgtccacg agacccccgc
181 gcgcagccat gagccccgcc ccccgctggt gcttgagag gggcgggacc tggagagagg
241 ctgctccgtg accccaccat gtctctcctt accacgagtt ccctggatac cccctgcctt
301 ggaaatggcc cccctcagcc tggcgcccc tcttcttcac ccactgtaaa ggaggagggt
361 ccggagccgt ggcccggggg tccggaccct gatgtcccag gcactgatga ggccagctca
421 gcctgcagca cagactgggt catcccagat cccgaagagg aaccagagcg caagcgaaag
481 aagggccccag ccccgaaagt gctgggccac gagctttgcc gtgtctgtgg ggacaaggcc
541 tccggcttcc actacaacgt gctcagctgc gaaggctgca agggcttctt ccggcgagct
601 gtggtccgtg gtggggccag gcgctatgcc tgccgggggt gcggaacctg ccagatggac
661 gctttcatgc ggcgcaagtg ccagcagctg cggctgcgca agtgcaagga ggcagggatg
721 agggagcagt gcgtcctttc tgaagaacag atccggaaga agaagattcg gaaacaacag
781 cagcagcagt cacagtcaca gtcgcagtca cctgtggggc cgcagggcag cagcagctca
841 gcctctgggc ctggggcttc ccctggtgga tctgaggcag gcagccaggg ctccggggaa
901 ggcgagggtg tccagctaac agcggctcaa gaactaatga tccagcagtt ggtggcggcc
961 caactgcagt gcaacaaacg ctcttcttcc gaccagccca aagtcacgcc ctggccccctg
1021 ggcgcagacc cccagtcccc agatgccccg cagcaacgct ttgcccactt cacggagctg
1081 gccatcatct cagtccagga gatcgtggac ttcgctaagc aagtgcctgg tttcctgcag
1141 ctggggccggg aggaccagat cgccctcctg aaggcatcca ctatcgagat catgctgcta

```

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1201 gagacagcca ggcgctacaa ccacgagaca gagtgtatca ccttcttgaa ggacttcacc
1261 tacagcaagg acgacttcca ccggtgcaggc ctgcagggtg agttcatcaa ccccatcttc
1321 gagttctcgc gggccatgcg gcggctgggc ctggacgacg ctgagtacgc cctgctcadc
1381 gccatcaaca tcttctcggc cgaccggccc aacgtgcagg agccgggccc cgtggaggcg
1441 ttgcagcagc cctacgtgga ggcgctgctg tcctacacgc gcatcaagag gccgcaggac
1501 cagctgcgct tcccgcgcat gctcatgaag ctggtgagcc tgcgcacgct gagctctgtg
1561 cactcggagc aggtcttcgc cttgcggctc caggacaaga agctgcccgc tctgctgtcg
1621 gagatctggg acgtccacga gtgaggggct ggccaccag cccacagcc ttgcctgacc
1681 accctccagc agatagacgc cggcaccct tcctcttcct agggtggaag gggccctggg
1741 ccgagcctgt agacctatcg gctctcatcc cttgggataa gcccagtcg aggtccagga
1801 ggctccctcc ctgcccagcg agtcttcag aaggggtgaa aggggtgcag gtcccacca
1861 ctgacccttc ccggtgccc tccctcccca gcttacacct caagcccagc acgcagtga
1921 ccttgaacag agggagggga ggacccatgg ctctccccc tagcccggga gaccaggggc
1981 cttcctcttc ctctgctttt atttaataaa aactaaaaac agaaaaaaa aaaaaaa

```

(SEQ ID NO:10)

In some embodiments, a nucleic acid encoding a polypeptide having the amino acid sequence of a murine liver X receptor alpha shown in Table 6 includes a coding sequence of the mRNA nucleic acid sequence disclosed in GenBank Acc. No. NM_013839, or a fragment thereof. In Table 12, the coding sequence extends from position 190 to position 1527.

Table 12. Polynucleotide sequence encoding murine liver X receptor alpha

```

1  gggaacgctg actctggagg ctgctgggat taggggtggg gtgactgaga agcagtcctt
61  ctgtcagagc aaagagcctc caggggtgagg agaggaagga gagagatgga actagaccgg
121 tctgcgggga aacgcgacag ttttggtaga gggacagtgt cttggtaatg tccagggtc
181 caggaagaga tgtccttgtg gctggaggcc tcaatgcctg atgtttctcc tgattctgca
241 acggagttgt ggaagacaga acctcaagat gcaggagacc agggaggcaa cacttgcadc
301 ctcaggaggg aagccaggat gcccagtcg actggggttg ctttagggat aggggtggag
361 tcagcagagc ctacagccct gctcccagg gcagagaccc tcccagagcc gacagagctt
421 cgtccacaaa agcggaaaaa gggcccagcc cccaaaatgc tggggaacga gctgtgcagt
481 gtctgtgggg acaaagcctc tggcttccat tacaacgtgc tgagctgcga gggctgcaag
541 ggattcttcc gccgcagtgt catcaaggga gcacgctatg tctgccacag cgggtggccac
601 tgcccatgga acacctacat gcggcggaat tgccaggagt gtcgacttcg caaatgccgc
661 caggcaggca tgaggaggga gtgtgtgctg tcagaagaac agatccgctt gaagaaactg
721 aagcggcaag aagaggaaca ggctcaagcc acttcgggtg ccccaagggt gtcctcacct
781 cctcaagtec tgccacagct cagcccagag cagctgggca tgatcgagaa gctggtggct
841 gccagcaac agtgtaacag gcgtcccttc tcagaccgcc tgcgctcac gccctggccc
901 attgcaccgc acctcagag ccgggaagcc cgacaacagc gctttgccca ctttactgag
961 ctggccatcg tgtccgtgca ggagattgtt gactttgcc aacagctccc tggcttccca
1021 cagctcagca gggaggacca gatcgccctg ctgaagacct ctgcaatcga ggtcatgctt
1081 ctggagacgt cacggaggta caaccccgcc agtgagagca tcaccttcct caaggacttc
1141 agttacaacc gggaagactt tgccaaagca gggctgcagg tggagttcat caaccccatc
1201 tttgagttct ccagagccat gaatgagctg caactcaatg atgctgagtt tgctctgctc
1261 attgccatca gcatcttctc tgcagaccgg cccaacgtgc aggaccagct ccaagtagag
1321 aggtgcaac acacatatgt ggaggccctg cagcctacg tctccatcaa ccacccccc
1381 gaccgactga tgttcccacg gatgctaatt aagctggtga gcttccgtac ttgagcagc
1441 gtccattcag agcaagtgtt tgcccttcgc ctgcaggaca aaaagcttcc ccctctgctg
1501 tctgagatct gggatgtcca cgagtgactg tttcacctg tcctttgtgt tggccacatg
1561 gcgaaggctc actgactgct tcccacgggt ggagcagact gagaagggca gacattcctg
1621 ggagctgggt gaaggagaga gccttgcgta gcattaaggg agagtcaaca ggttgggtgt
1681 tttctggctg ctgggcagtt gggatctact aacgttgtat accatctgaa gaccttgttg

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1741 acccaaccaa ata

(SEQ ID NO:11)

In some embodiments, a nucleic acid encoding a polypeptide having the amino acid sequence of a murine liver X receptor beta shown in Table 7 includes a coding sequence of the mRNA nucleic acid sequence disclosed in GenBank Acc. No. NM_009473, or a fragment thereof.

In Table 13, the coding sequence extends from position 271 to position 1611.

Table 13. Polynucleotide sequence encoding murine liver X receptor beta

1	ggcgaagtta	cttttgcttt	tcgctcagca	agcgtctgtt	cttcgagcta	ctcccaggct
61	tctgaagtta	cttccaaagt	gctgtggagg	cacaatcacc	ggtgcggaca	cagagggaac
121	tctgcctcc	cacggccgtt	tccagggcaa	cagagtcgga	gacccccctg	gacccccctc
181	ccgatcgccg	gtgcagtcat	gagccccgcc	tccccctggt	gcacggagag	ggcgggggcc
241	tggaacaagc	aggctgcttc	gtgaccact	atgtcttccc	ccacaagttc	tctggacact
301	cccggtgcctg	ggaatggttc	tcctcagccc	agtacctccg	ccacgtcacc	cactattaag
361	gaagaggggc	aggagactga	tcctcctcca	ggctctgaag	ggtccagctc	tgcctacatc
421	gtggtcatct	tagagccaga	ggatgagcct	gagcgcaagc	ggaagaaggg	gccggccccg
481	aagatgctgg	gccatgagct	gtgccgcgtg	tgccggagaca	aggcctcggg	cttccactac
541	aacgtgctca	gctgtgaagg	ctgcaaaggc	ttcttccggc	gcagtgtggt	ccacggtggg
601	gccggggcgt	atgcctgtcg	gggcagcgga	acctgccaga	tggatgcctt	catgcggcgc
661	aagtgccagc	tctgccggct	gcgcaagtgc	aaggaggctg	gcatgcggga	gcagtgcgtg
721	ctctctgagg	agcagattcg	gaagaaaagg	attcagaagc	agcaacagca	gcagccacca
781	cccccatctg	agccagcagc	cagcagctca	ggccggccag	cggcctcccc	tggcacttcg
841	gaagcaagca	gccagggctc	cggggaagga	gagggcatcc	agctgaccgc	ggctcaggag
901	ctgatgatcc	agcagttagt	tgccgcgcag	ctgcagtgca	acaaacgac	tttctccgac
961	cagcccaaag	tcacgccctg	gcccctgggt	gcagaccctc	agtcccagga	tgcccgtcag
1021	caacgctttg	cccacttcac	cgagctagcc	atcatctcgg	tccaggagat	tgtggacttt
1081	gccaaagcag	tgccagggtt	cttgcaattg	ggccgggagg	accagatcgc	cctcctgaag
1141	gcgtccacca	ttgagatcat	gttgctagaa	acagccagac	gctacaacca	cgagacagaa
1201	tgcatcacgt	tcctgaagga	cttcacctac	agcaaggacg	acttccaccg	tgcaggcttg
1261	caggtggaat	tcataaatcc	catcttcgag	ttctcgcggg	ccatgcggcg	gctggccttg
1321	gacgatgcag	agtatgcctt	gcttatcgcc	atcaacatct	tctcagccga	tgggccta
1381	gtgcaggagc	ccagccgtgt	ggaggccctg	cagcagccat	acgtggaggc	gctcctctcc
1441	tacacgagga	tcaagcggcc	acaggaccag	ctccgcttcc	cacgcatgct	catgaagctg
1501	gtgagcctgc	gcaccctcag	ctccgtgcac	tcggagcagg	tctttgcatt	gcgactccag
1561	gacaagaagc	tgccggccctt	gctgtccgag	atctgggatg	tgcacgagta	ggggcagcca
1621	caagtgcctc	agccttggtg	gtgtcttctt	gaagatggac	tcttcacctc	tcctcctggg
1681	gtgggaggac	attgtcacgg	cccagtcctt	cgggctcagc	ctcaaactca	gcggcagttg
1741	gcactagaag	gccccacccc	accattgag	tcttccaaga	gtggtgaggg	tcacaggtcc
1801	tagcctctga	ccgttcccag	ctgccctccc	acccacgctt	acacctcagc	ctaccacacc
1861	atgcaccttg	agtggagaga	ggttagggca	ggtggccccc	cacagttggg	agaccacagg
1921	ccctctcttc	tgcccttttt	atttaataaa	aaaacaaaaa	taaa	

(SEQ ID NO:12)

Additionally, the invention includes polynucleotides that are mutant or variant nucleic acids of the sequences shown in Tables 8-13, or a fragment thereof, any of whose bases may be changed from the disclosed sequence while still encoding a polypeptide that maintains its SIRT1 protein-like activities and physiological functions. An SIRT1 mutant or variant polynucleotide encodes a mutant or variant SIRT1 or LXR polypeptide that may have

from 1 amino acid residue up to 1% of the residues changed, or up to 2%, or up to 5%, or up to 8%, or up to 10%, or up to 15%, or up to 20%, or somewhat higher percent, of the residues changed from a wild type or reference sequence. By “nucleic acid” or “polynucleotide” is meant a DNA, an RNA, a DNA or RNA including one or more modified nucleotides or modified pentose phosphate backbone structures, a polypeptide-nucleic acid, and similar constructs that preserve the coding properties of the sequence of bases included in the construct. The invention further includes the complement of the nucleic acid sequence of any SIRT1 or LXR encoding sequence, including fragments, derivatives, analogs and homolog thereof. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications.

Also included are nucleic acid fragments. A nucleic acid fragment may encode a fragment of an SIRT1 or LXR polypeptide. In addition SIRT1 or LXR nucleic fragments may be used as hybridization probes to identify SIRT1 or LXR protein-encoding nucleic acids (*e.g.*, SIRT1 or LXR mRNA) and fragments for use as polymerase chain reaction (PCR) primers for the amplification or mutation of SIRT1 or LXR nucleic acid molecules. As used herein, the term “nucleic acid molecule” is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

Nucleic Acids

As used herein, a “nucleic acid” or “polynucleotide”, and similar terms and phrases, relate to polymers composed of naturally occurring nucleotides as well as to polymers composed of synthetic or modified nucleotides. Thus, as used herein, a polynucleotide that is a RNA, or a polynucleotide that is a DNA, or a polynucleotide that contains both deoxyribonucleotides and ribonucleotides, may include naturally occurring moieties such as the naturally occurring bases and ribose or deoxyribose rings, or they may be composed of synthetic or modified moieties such as those described below. A polynucleotide employed in the invention may be single stranded or it may be a base paired double stranded structure, or even a triple stranded base paired structure.

Nucleic acids and polynucleotides may be 20 or more nucleotides in length, or 30 or more nucleotides in length, or 50 or more nucleotides in length, or 100 or more, or 1000 or

more, or tens of thousands or more, or hundreds of thousands or more, in length. As used herein, “oligonucleotides” and similar terms based on this relate to short polymers composed of naturally occurring nucleotides as well as to polymers composed of synthetic or modified nucleotides, as described in the immediately preceding paragraph. Oligonucleotides may be 10 or more nucleotides in length, or 15, or 16, or 17, or 18, or 19, or 20 or more nucleotides in length, or 21, or 22, or 23, or 24 or more nucleotides in length, or 25, or 26, or 27, or 28 or 29, or 30 or more nucleotides in length, 35 or more, 40 or more, 45 or more, up to about 50, nucleotides in length. Oligonucleotides may be chemically synthesized and may be used as siRNAs, PCR primers, or probes.

It is understood that, because of the overlap in size ranges provided in the preceding paragraph, the terms “polynucleotide” and “oligonucleotide” may be used synonymously herein.

As used herein “nucleotide sequence”, “oligonucleotide sequence” or “polynucleotide sequence”, and similar terms, relate interchangeably both to the sequence of bases that an oligonucleotide or polynucleotide has, as well as to the oligonucleotide or polynucleotide structure possessing the sequence. A nucleotide sequence or a polynucleotide sequence furthermore relates to any natural or synthetic polynucleotide or oligonucleotide in which the sequence of bases is defined by description or recitation of a particular sequence of letters designating bases as conventionally employed in the field.

A “nucleoside” is conventionally understood by workers of skill in fields such as biochemistry, molecular biology, genomics, and similar fields related to the field of the invention as comprising a monosaccharide linked in glycosidic linkage to a purine or pyrimidine base; and a “nucleotide” comprises a nucleoside with at least one phosphate group appended, typically at a 3' or a 5' position (for pentoses) of the saccharide, but may be at other positions of the saccharide. Nucleotide residues occupy sequential positions in an oligonucleotide or a polynucleotide. A modification or derivative of a nucleotide may occur at any sequential position in an oligonucleotide or a polynucleotide. All modified or derivatized oligonucleotides and polynucleotides are encompassed within the invention and fall within the scope of the claims. Modifications or derivatives can occur in the phosphate group, the monosaccharide or the base.

By way of nonlimiting examples, the following descriptions provide certain modified or derivatized nucleotides, all of which are within the scope of the polynucleotides of the invention. The monosaccharide may be modified by being, for example, a pentose or a

hexose other than a ribose or a deoxyribose. The monosaccharide may also be modified by substituting hydroxyl groups with hydro or amino groups, by alkylating or esterifying additional hydroxyl groups, and so on. Substituents at the 2' position, such as 2'-O-methyl, 2'-O-ethyl, 2'-O-propyl, 2'-O-allyl, 2'-O-aminoalkyl or 2'-deoxy-2'-fluoro group provide enhanced hybridization properties to an oligonucleotide.

The bases in oligonucleotides and polynucleotides may be "unmodified" or "natural" bases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). In addition they may be bases with modifications or substitutions. Nonlimiting examples of modified bases include other synthetic and natural bases such as hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine, 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, and 2-propyl and other alkyl derivatives of adenine and guanine

The linkages between nucleotides is commonly the 3'-5' phosphate linkage, which may be a natural phosphodiester linkage, a phosphothioester linkage, and still other synthetic linkages. Oligonucleotides containing phosphorothioate backbones have enhanced nuclease stability. Nonlimiting examples of modified backbones include, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates and boranophosphates

Any modifications including those exemplified in the above description can readily be incorporated into, and are comprised within the scope of, the polynucleotides of the invention. Use of any modified nucleotide is equivalent to use of a naturally occurring nucleotide having the same base-pairing properties, as understood by a worker of skill in the art. All equivalent modified nucleotides fall within the scope of the present invention as disclosed and claimed herein.

An "isolated" nucleic acid molecule is one that is separated from other nucleic acid molecules that are present in the natural source of the nucleic acid. Examples of isolated nucleic acid molecules include, but are not limited to, recombinant DNA molecules contained in a vector, recombinant DNA molecules maintained in a heterologous host cell, partially or substantially purified nucleic acid molecules, and synthetic DNA or RNA molecules.

A nucleic acid molecule disclosed herein, *e.g.*, a nucleic acid molecule having the nucleotide sequence of Table 8-13, or a complement thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of Table 8-13 as a hybridization probe, SIRT1 or LXR nucleic acid sequences can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook *et al.*, eds., *MOLECULAR CLONING: A LABORATORY MANUAL* 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, eds., *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY*, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid disclosed in the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to SIRT1 or LXR nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

Synthesis of Polynucleotides

Oligonucleotides and polynucleotides can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer. Methods for synthesizing oligonucleotides include well-known chemical processes, including, but not limited to, sequential addition of nucleotide phosphoramidites onto surface-derivatized particles, as described by T. Brown and Dorcas J. S. Brown in *Oligonucleotides and Analogues A*

Practical Approach, F. Eckstein, editor, Oxford University Press, Oxford, pp. 1-24 (1991), and incorporated herein by reference.

An example of a synthetic procedure uses Expedite RNA phosphoramidites and thymidine phosphoramidite (Proligo, Germany). Synthetic oligonucleotides are deprotected and gel-purified (Elbashir et al. (2001) *Genes & Dev.* 15, 188-200), followed by Sep-Pak C18 cartridge (Waters, Milford, Mass., USA) purification (Tuschl et al. (1993) *Biochemistry*, 32:11658-11668). Complementary ssRNAs are incubated in an annealing buffer (100 mM potassium acetate, 30 mM HEPES-KOH at pH 7.4, 2 mM magnesium acetate) for 1 min at 90° C followed by 1 h at 37° C to hybridize to the corresponding ds-polynucleotides.

Other methods of oligonucleotide synthesis include, but are not limited to solid-phase oligonucleotide synthesis according to the phosphotriester and phosphodiester methods (Narang, et al., (1979) *Meth. Enzymol.* 68:90), and to the H-phosphonate method (Garegg, P. J., et al., (1985) "Formation of internucleotidic bonds via phosphonate intermediates", *Chem. Scripta* 25, 280-282; and Froehler, B. C., et al., (1986a) "Synthesis of DNA via deoxynucleoside H-phosphonate intermediates", *Nucleic Acid Res.*, 14, 5399-5407, among others) and synthesis on a support (Beaucage, et al. (1981) *Tetrahedron Letters* 22:1859-1862) as well as phosphoramidate techniques (Caruthers, M. H., et al., "Methods in Enzymology," Vol. 154, pp. 287-314 (1988), U. S. Patent 5,153,319, 5,132,418, 4,500,707, 4,458,066, 4,973,679, 4,668,777, and 4,415,732, and others described in "Synthesis and Applications of DNA and RNA," S. A. Narang, editor, Academic Press, New York, 1987, and the references contained therein, and nonphosphoramidite techniques. Solid phase synthesis helps isolate the oligonucleotide from impurities and excess reagents. Once cleaved from the solid support the oligonucleotide may be further isolated by known techniques.

Vectors

The present invention provides various vectors that contain one or more polynucleotides of the invention. Advantageously any vector disclosed in the invention includes a promoter, an enhancer, or both, operably linked to the nucleotide sequence.

Methods for preparing the vectors disclosed in the invention are widely known in the fields of molecular biology, cell biology, oncology and related fields of medicine, and other fields related to the present invention. Methods useful for preparing the vectors are described, by way on nonlimiting example, in *Molecular Cloning: A Laboratory Manual* (3rd Edition) (Sambrook, J et al. (2001) Cold Spring Harbor Laboratory Press, Cold Spring

Harbor, NY), and Short protocols in molecular biology (5th Ed.) (Ausubel FM et al. (2002) John Wiley & Sons, New York City).

Antibodies

The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab}, F_{ab}' and F_{(ab')₂} fragments, and an F_{ab} expression library. In general, antibody molecules obtained from humans relates to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG₁, IgG₂, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of human antibody species. Any antibody disclosed herein binds "immunospecifically" to its cognate antigen. By immunospecific binding is meant that an antibody raised by challenging a host with a particular immunogen binds to a molecule such as an antigen that includes the immunogenic moiety with a high affinity, and binds with only a weak affinity or not at all to non-immunogen-containing molecules. As used in this definition, high affinity means having a dissociation constant less than about 1×10^{-6} M, and weak affinity means having a dissociation constant higher than about 1×10^{-6} M.

An isolated protein of the invention, or a complex containing such a protein, intended to serve as an antigen, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that immunospecifically bind the antigen, using standard techniques for polyclonal and monoclonal antibody preparation. The full-length protein can be used or, alternatively, the invention provides antigenic peptide fragments of the antigen for use as immunogens. An antigenic peptide fragment comprises at least 6 amino acid residues of the amino acid sequence of the full length protein, such as an amino acid sequence shown in Tables 2-7, and encompasses an epitope thereof such that an antibody raised against the peptide forms a specific immune complex with the full length protein or with any fragment that contains the epitope. Preferably, the antigenic peptide comprises at least 10 amino acid residues, or at least 15 amino acid residues, or at least 20 amino acid residues, or at least 30

amino acid residues. Preferred epitopes encompassed by the antigenic peptide are regions of the protein that are located on its surface; commonly these are hydrophilic regions.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of the SIRT1 or LXR protein that is located on the surface of the protein, *e.g.*, a hydrophilic region. A hydrophobicity analysis of the human SIRT1 or LXR protein sequence will indicate which regions of a growth promoting polypeptide are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, *e.g.*, Hopp and Woods, 1981, *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle 1982, *J. Mol. Biol.* 157: 105-142, each incorporated herein by reference in their entirety. Antibodies that are specific for one or more domains within an antigenic protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

A protein of the invention, or a derivative, fragment, analog, homolog or ortholog thereof, may be utilized as an immunogen in the generation of antibodies that immunospecifically bind these protein components.

Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies directed against a protein of the invention, or against derivatives, fragments, analogs homologs or orthologs thereof (see, for example, *Antibodies: A Laboratory Manual*, Harlow E, and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference). Some of these antibodies are discussed below.

Polyclonal Antibodies

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by one or more injections with the native protein, a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, the naturally occurring immunogenic protein, a chemically synthesized polypeptide representing the immunogenic protein, or a recombinantly expressed immunogenic protein. Furthermore, the protein may be conjugated to a second protein known to be immunogenic in the mammal being immunized. Examples

of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. The preparation can further include an adjuvant.

The polyclonal antibody molecules directed against the immunogenic protein can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as affinity chromatography using protein A or protein G, or on a column of the antigen. Purification of immunoglobulins is discussed, for example, by D. Wilkinson (The Scientist, published by The Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28).

Monoclonal Antibodies

Monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized in vitro.

The immunizing agent will typically include the protein antigen, a fragment thereof or a fusion protein thereof. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the antigen. After the desired hybridoma cells are identified, the clones can be subcloned by limiting dilution procedures and grown by standard methods (Goding, 1986). Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown in vivo as ascites in a mammal.

The monoclonal antibodies secreted by the subclones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

Recombinant Vectors and Host Cells

Certain aspects of the invention disclose methods of promoting ABCA1-mediated cholesterol efflux from a mammalian cell that include the introduction into the cell a nucleic acid that contains a sequence encoding a protein deacetylase. The mammalian cell may be a human cell. Various the protein deacetylase may be a eukaryotic Sir2 protein, or a mammalian SIRT1 protein. Techniques for transfecting mammalian cells with intended nucleic acid sequences are widely known to workers of skill in the field of the invention. Certain nonlimiting examples of such methods are described in detail in the following

One aspect disclosed in the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors disclosed in the invention comprise a nucleic acid disclosed in the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is

introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY* 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors disclosed in the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, SIRT1 or LXR proteins, mutant forms of the SIRT1 or LXR protein, fusion proteins, etc.).

The recombinant expression vectors disclosed in the invention can be designed for expression of the SIRT1 or LXR protein in prokaryotic or eukaryotic cells. For example, the SIRT1 or LXR protein can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY* 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (1) to increase expression of recombinant protein; (2) to increase the solubility of the recombinant protein; and (3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann et al., (1988) *Gene* 69:301-315) and pET 11d (Studier et al., *GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY* 185, Academic Press, San Diego, Calif. (1990) 60-89).

In another embodiment, the proteins disclosed in the invention are expressed using a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, et al., (1987) *EMBO J* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz et al., (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego, Calif.).

Alternatively, the proteins disclosed in the complexes of the invention can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., SF9 cells) include the pAc series (Smith et al. (1983) *Mol Cell Biol* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid disclosed in the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells. See, e.g., Chapters 16 and 17 of Sambrook *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL*. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv Immunol* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and

Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev* 3:537-546).

Another aspect disclosed in the invention pertains to host cells into which a recombinant expression vector disclosed in the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, the SIRT1 or LXR protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (2001), Ausubel *et al.* (2002), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding the growth promoter or can be introduced on a separate vector. Cells stably

transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) the SIRT1 or LXR protein. Accordingly, the invention further provides methods for producing the SIRT1 or LXR protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding the SIRT1 or LXR protein has been introduced) in a suitable medium such that the SIRT1 or LXR protein is produced. In another embodiment, the method further comprises isolating the SIRT1 or LXR protein from the medium or the host cell.

Transfection of a vertebrate cell can further be accomplished using recombinant vectors which include, but are not limited, to adenovirus, adeno-associated virus, and retrovirus vectors, in addition to other particles that introduce DNA into cells, such as liposomes. Techniques such as those described above can be utilized for the introduction of any SIRT1 or LXR polypeptide encoding nucleotide sequences into vertebrate cells. For example, for transfection of mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the SIRT1 or LXR nucleotide sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing an SIRT1 or LXR product in infected hosts (*e.g.*, See Logan & Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:3655-3659). In cases where only a portion of an SIRT1 or LXR coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (See Bitter et al., 1987, *Methods in Enzymol.* 153:516-544).

Therapeutic Treatment

Certain pathologies and medical conditions are believed to respond favorably to the expression of a heterologous SIRT1 or LXR or a heterologous LXR α or LXR β in the cells of

a subject. Accordingly, the present invention discloses a method of treating a pathology, a disease or a medical condition in a subject, wherein the pathology responds to a SIRT1 or LXR, LXR α or LXR β polypeptide. The method includes administering a nucleic acid encoding an SIRT1 or LXR, LXR α or LXR β polypeptide to the subject in an amount effective to attenuate or ameliorate the pathology. Attenuating a pathology signifies that a trend of worsening symptomology is abated to a slower or more gentle trend of worsening. Ameliorating a pathology signifies an actual improvement in the patient, such that the signs and indications of the pathology diminish, and the patient improves toward better health. In important implementations of this method the pathology is chosen from among myocardial infarction, cerebrovascular stroke, a kidney disease, a neurological disease including Alzheimer's disease, and the like. In advantageous embodiments of the method of treating a pathology the subject is a human.

In various embodiments of the methods of treatment described herein, a nucleic acid encoding an SIRT1 or LXR, LXR α or LXR β polypeptide, a variant thereof, or a fragment thereof, may be administered to a subject in any of a variety of compositions that ensure efficient delivery of the nucleic acid sequence into cells, including delivery into the cells of a subject.

Treatment of a subject with an *SIRT1* or *LXR* nucleic acid sequence can be accomplished by administering a suitable nucleic acid, plasmid, vector, viral vector, liposomal or similar composition that is effective to introduce the *SIRT1* or *LXR* nucleic acid sequence into a vertebrate cell. Transfection of nucleic acids may be assisted with the use of cationic amphiphiles (US Patent 6,503,945 and references disclosed therein). Ex vivo retroviral gene therapy is described, for example, in Hacein-Bey-Abina et al. (2003, Science302:415-419). Methods for therapeutic introduction of a transgene into a subject are discussed in "Gene Transfer Methods: Introducing DNA Into Living Cells and Organisms" P.A. Norton and L.F. Steel, Eaton Publishing, 2000. Approaches to the therapeutic introduction of transgenes into cells and organisms are provided in "Gene Therapy Protocols" Paul D. Robbins (Ed.), Humana Press (1997).

Pharmaceutical Compositions Comprising Polynucleotides

Pharmaceutical compositions for therapeutic applications include one or more polynucleotides and a carrier. The pharmaceutical composition comprising the one or more polynucleotide is useful for treating a disease or disorder associated with the expression or

activity of a Target Gene. Carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. For drugs administered orally, pharmaceutically acceptable carriers include, but are not limited to, pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservatives.

A maximum dosage of 5 mg polynucleotide per kilogram body weight of recipient per day is sufficient to inhibit or completely suppress expression of the target gene. In general, a suitable dose of polynucleotide will be in the range of 0.01 to 5.0 milligrams per kilogram body weight of the recipient per day, preferably in the range of 0.1 to 200 micrograms per kilogram body weight (mcg/kg) per day, more preferably in the range of 0.1 to 100 mcg/kg per day, even more preferably in the range of 1.0 to 50 mcg/kg per day, and most preferably in the range of 1.0 to 25 mcg/kg per day. The pharmaceutical composition may be administered once daily, or the polynucleotide may be administered as two, three, four, five, six or more sub-doses at appropriate intervals throughout the day. In that case, the polynucleotide contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded as a sustained release formulation for delivery over several days, e.g., using a conventional formulation which provides sustained release of the polynucleotide over a several day period. Sustained release formulations are well known in the art. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and in vivo half-lives for the individual polynucleotides encompassed by the invention can be made using conventional methodologies or on the basis of in vivo testing using an appropriate animal model, and can be adjusted during treatment according to established criteria for determining appropriate dose-response characteristics.

The pharmaceutical compositions encompassed by the invention may be administered by any means known in the art including, but not limited to oral or parenteral routes, including intravenous, intramuscular, intraperitoneal, subcutaneous, transdermal, airway (aerosol), rectal, vaginal and topical (including buccal and sublingual) administration. In

certain embodiments, the pharmaceutical compositions are administered by intravenous or intraparenteral infusion or injection, and in additional common embodiments the pharmaceutical composition comprising polynucleotides may be delivered directly in situ to a tumor, a cancer or a precancerous growth using laparoscopic and similar microsurgical procedures.

For intramuscular, intraperitoneal, subcutaneous and intravenous use, the pharmaceutical compositions of the invention will generally be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity. Suitable aqueous vehicles include Ringer's solution and isotonic sodium chloride. In a preferred embodiment, the carrier consists exclusively of an aqueous buffer. In this context, "exclusively" means no auxiliary agents or encapsulating substances are present which might affect or mediate uptake of polynucleotide in the cells that express the target gene. Such substances include, for example, micellar structures, such as liposomes or capsids, as described below. Surprisingly, the present inventors have discovered that compositions containing only naked polynucleotide and a physiologically acceptable solvent are taken up by cells, where the polynucleotide effectively inhibits expression of the target gene. Although microinjection, lipofection, viruses, viroids, capsids, capsoids, or other auxiliary agents are required to introduce polynucleotide into cell cultures, surprisingly these methods and agents are not necessary for uptake of polynucleotide in vivo. Aqueous suspensions according to the invention may include suspending agents such as cellulose derivatives, sodium alginate, polyvinyl-pyrrolidone and gum tragacanth, and a wetting agent such as lecithin. Suitable preservatives for aqueous suspensions include ethyl and n-propyl p-hydroxybenzoate.

The pharmaceutical compositions useful according to the invention also include encapsulated formulations to protect the polynucleotide against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for

example, as described in U.S. Pat. No. 4,522,811; PCT publication WO 91/06309; and European patent publication EP-A-43075, which are incorporated by reference herein.

In certain embodiments, the encapsulated formulation comprises a viral coat protein. In this embodiment, the polynucleotide may be bound to, associated with, or enclosed by at least one viral coat protein. The viral coat protein may be derived from or associated with a virus, such as a polyoma virus, or it may be partially or entirely artificial. For example, the coat protein may be a Virus Protein 1 and/or Virus Protein 2 of the polyoma virus, or a derivative thereof.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds which exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosages for use in humans. The dosage of compositions of the invention lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays and animal models to achieve a circulating plasma concentration range of the compound that includes the IC₅₀ (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans.

In addition to their administration individually or as a plurality, as discussed above, the polynucleotides useful according to the invention can be administered in combination with other known agents effective in treatment of diseases. In any event, the administering physician can adjust the amount and timing of polynucleotide administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

Pharmaceutical Compositions

The polynucleotides disclosed in the invention are designated "active compounds" or "therapeutics" herein. These therapeutics can be incorporated into pharmaceutical compositions suitable for administration to a subject.

The invention includes methods for increasing the ratio of cholesterol bound to high density lipoprotein (HDL) to total cholesterol in the plasma of a mammal by administering an agent that stimulates SIRT1 activity, as well as use of an agent that stimulates SIRT1 activity in the manufacture of a medicament for increasing the ratio of cholesterol bound to high density lipoprotein (HDL) to total cholesterol in the plasma of a mammal by administering the agent to the mammal. Such agents are designated "active compounds" or "therapeutics" herein. An example of such an agent is T0901317. These therapeutics can be incorporated into pharmaceutical compositions suitable for administration to a subject.

The invention also includes methods for increasing the ratio of cholesterol bound to high density lipoprotein (HDL) to total cholesterol in the plasma of a mammal by administering an agent that promotes formation of a complex comprising a mammalian SIRT1 protein and a mammalian LXR protein. In addition the invention discloses the use of an agent that promotes formation of a complex comprising a mammalian SIRT1 protein and a mammalian LXR protein in the manufacture of a medicament for increasing the ratio of cholesterol bound to high density lipoprotein (HDL) to total cholesterol in the plasma of a mammal by administering the agent to the mammal. Such agents are designated "active compounds" or "therapeutics" herein. Examples of such agents include either 22(R)-hydroxycholesterol, or 9-cis retinoic acid, or combinations of both 22(R)-hydroxycholesterol and 9-cis retinoic acid. These therapeutics can be incorporated into pharmaceutical compositions suitable for administration to a subject.

As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in textbooks such as Remington's Pharmaceutical Sciences, Gennaro AR (Ed.) 20th edition (2000) Williams & Wilkins PA, USA, and Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, by Delgado and Remers, Lippincott-Raven., which are incorporated herein by reference. Preferred examples of components that may be used in such carriers or diluents include, but are not limited to, water, saline, phosphate salts, carboxylate salts, amino acid solutions, Ringer's solutions, dextrose (a synonym for glucose) solution, and 5% human serum albumin. By way of nonlimiting example, dextrose may be used as 5% or 10% aqueous solutions. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents

for pharmaceutically active substances is well known in the art. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral, nasal, inhalation, transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intravenous, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release pharmaceutical active agents over shorter time periods. Advantageous polymers are biodegradable, or biocompatible. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as

described in U.S. Pat. No. 4,522,811. Sustained-release preparations having advantageous forms, such as microspheres, can be prepared from materials such as those described above.

The polynucleotides disclosed in the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by any of a number of routes, *e.g.*, as described in U.S. Patent Nos. 5,703,055. Delivery can thus also include, *e.g.*, intravenous injection, local administration (see U.S. Pat. No. 5,328,470) or stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a kit, *e.g.*, in a container, pack, or dispenser together with instructions for administration.

Also within the invention is the use of a therapeutic in the manufacture of a pharmaceutical composition or medicament for treating a respiratory viral infection in a subject.

In several embodiments the polynucleotides disclosed in the invention are delivered by liposome-mediated transfection, for example by using commercially available reagents or techniques, *e.g.*, Oligofectamine™, LipofectAmine™ reagent, LipofectAmine 2000™ (Invitrogen), as well as by electroporation, and similar techniques. Additionally polynucleotides are delivered to animal models, such as rodents or non-human primates, through inhalation and instillation into the respiratory tract. Additional routes for use with animal models include intravenous (IV), subcutaneous (SC), and related routes of administration. The pharmaceutical compositions include additional components that protect the stability of polynucleotides, prolong their lifetime, potentiate their function, or target them to specific tissues/cells. These include a variety of biodegradable polymers, cationic polymers (such as polyethyleneimine), cationic copolypeptides such as histidine-lysine (HK) polypeptides see, for example, PCT publications WO 01/47496 to Mixson *et al.*, WO 02/096941 to Biomerieux, and WO 99/42091 to Massachusetts Institute of Technology), PEGylated cationic polypeptides, and ligand-incorporated polymers, etc. positively charged polypeptides, PolyTran polymers (natural polysaccharides, also known as scleroglucan; Pillsbury Co., Minneapolis, Minn), surfactants (Infasurf; Forest Laboratories, Inc.; ONY

Inc.), and cationic polymers (such as polyethyleneimine). Infasurf® (calfactant) is a natural lung surfactant isolated from calf lung for use in intratracheal instillation; it contains phospholipids, neutral lipids, and hydrophobic surfactant-associated proteins B and C. The polymers can either be uni-dimensional or multi-dimensional, and also could be microparticles or nanoparticles with diameters less than 20 microns, between 20 and 100 microns, or above 100 micron. The said polymers could carry ligand molecules specific for receptors or molecules of special tissues or cells, thus be used for targeted delivery of polynucleotides. The polynucleotides are also delivered by cationic liposome based carriers, such as DOTAP, DOTAP/Cholesterol (Qbiogene, Inc.) and other types of lipid aqueous solutions. In addition, low percentage (5-10%) glucose aqueous solution, and Infasurf are effective carriers for airway delivery of polynucleotides.

Gene Therapy

Currently gene therapy is in a high state of development for use in therapeutic and prophylactic (e. g. immunizing) settings. Novel delivery methods are available, and several clinical trials of various gene therapeutic modalities are under way. U. S. Patent Application Publication 20060115462 entitled "Direct DNA delivery to bone cells" discloses a method that enables in vivo delivery of a polynucleotide such as a naked polynucleotide or a gene expression vector to cells of a mammalian bone limb.

U. S. Patent Application Publication 20060115456 entitled "Replication-competent adenoviral vectors" discloses improved replication-competent adenoviral vectors, including adenovirus types 2, 4, 5, and 7, for use in delivery of nucleic acids and polynucleotides for therapeutic and prophylactic applications.

U. S. Patent Application Publication 20060104950 entitled "Methods of Transducing genes into T cells" discloses transduction of a gene into activated T cells using a paramyxovirus vector.

U. S. Patent Application Publication 20060110361 entitled "Methods of making viral particles having a modified cell binding activity and uses thereof" discloses a method for packaging viral particles such that one or more peptides on the surface of the article are derived from the packaging cell. The application states that such a system is of use, for example, in gene therapy treatments.

U. S. Patent Application Publication 20060110364 entitled "Vector-mediated delivery of polynucleotides encoding soluble VEGF receptors" discloses vector compositions for

expression of a soluble form of VEGFR3 and methods for their use in the treatment of cancer.

U. S. Patent Application Publication 20050239204 entitled "Multifunctional molecular complexes for gene transfer to cells" discloses a non-viral multifunctional molecular complex for the transfer of a nucleic acid composition to a target cell.

VandenDriessche et al. showed that hemophilia A could be effectively treated by an in vivo gene therapy that introduces the human Factor VIII gene into Factor VIII-deficient mice by means of retroviral vectors.⁷⁹ Gene transfer occurred at least in liver, spleen and lungs. The safety of a nonviral somatic cell gene therapy system in patients with severe hemophilia A has been tested by Roth et al.⁸⁰

Because of its stable, post-mitotic state skeletal muscle is an important target for genetic manipulation by use of both integrating and non-integrating viral and non-viral vectors. Muscle-directed gene therapy to prevent autoimmune diabetes in nonobese diabetic (NOD) mice was reported by Goudy et al.⁸¹ They developed recombinant adeno-associated viral vectors containing murine cDNAs for immunomodulatory cytokines IL4 or IL10. Skeletal muscle transduction of female NOD mice with IL10, but not IL4, completely abrogated diabetes.

According to Wells⁸², adeno-associated viral vectors and naked plasmid DNA are currently the vectors of choice for gene transfer into muscle. Major breakthroughs in the field of vector delivery systems, particularly those using the vascular route have been generated within last two years or so, such that gene therapy of muscular dystrophies and the use of muscle as a platform for the production of secreted proteins has become a clinical possibility.

Oncolytic therapy is a novel anticancer treatment based on attenuated lytic viruses such as adenovirus (Ad).⁸³ These viruses induce destruction of host cells by lysis and are thus distinct from classical gene therapy viruses. Oncolytic Ads are genetically engineered so as to replicate only in cancer cells. Three oncolytic Ads have completed Phase I and II clinical trials in cancer patients. These viruses are being engineered further to provide them with additional therapeutic genes.⁸³

Takahashi et al. have started a clinical study of multidrug resistance (MDR1) gene therapy. The two patients treated to date are in complete remission and suffered no apparent adverse effects from the MDR1 gene transfer.⁸⁴

Broedl et al. describe that, in the face of currently ineffective treatments in various hyperlipidemias, somatic gene therapy is considered to be a potential approach to therapy. In many cases preclinical proof-of-principle studies have already been performed, and in the case homozygous familial hypercholesterolaemia a clinical trial has been conducted.⁸⁵

Antisense oligodeoxynucleotides directed against clusterin have been used in a Phase I clinical trial for treatment of prostate cancer.⁸⁶ The treatment provided up to 90% suppression of clusterin in prostate cancer. Miyake et al. state that phase II studies were scheduled to begin in early 2005.⁸⁶

Satoh et al. review the status of prostate cancer gene therapy, including virus-mediated transduction of the herpes simplex virus thymidine kinase gene followed by a course of the prodrug ganciclovir.⁸⁷ They report that a gene therapy clinical trial for human prostate cancer demonstrated safety, clinical efficacy, and biological effects of antitumor activity. According to Satoh et al., after numerous favorable preclinical studies several clinical studies have been approved for gene therapy of prostate cancer using immunomodulatory cytokines, such as interleukin-2, interleukin-12, B7-1 (CD80), B7-2 (CD86) and granulocyte-macrophage colony-stimulating factor.⁸⁷

Assay Methods for Identifying Potential Active Agents

In various aspects the invention discloses assay methods intended to determine whether a candidate chemical compound or agent modulates an activity of interest in the present invention. Such methods include the following.

The invention discloses a method for assessing whether a candidate substance modulates an LXR-dependent process, including

- a) transfecting a cell with a vector harboring a reporter gene operably driven by an LXRE;
- b) contacting the cell with the candidate; and
- c) determining whether the candidate modulates the expression of the reporter gene in comparison with a cell not contacted with the candidate;

whereby a difference in extent of occurrence of the LXR-dependent process detected between the presence and absence of the candidate indicates that the candidate modulates the LXR-dependent process.

The invention additionally discloses a method for assessing whether a candidate substance modulates an SIRT1-dependent effect of an LXR, including

- a) transfecting a cell with a vector harboring an SIRT1 gene;
- b) further transfecting the cell with a vector harboring a reporter gene operably driven by an LXRE promoter;
- c) contacting the cell with the candidate; and
- d) determining whether the candidate modulates the expression of the reporter gene in comparison with a cell not contacted with the candidate;

whereby a difference in the extent of the SIRT1-dependent effect of the LXR detected between the presence and absence of the candidate indicates that the candidate modulates the SIRT1-dependent effect of an LXR.

The invention further discloses a method for assessing whether a candidate substance modulates the formation of a specific binding pair comprising specific binding pair members SIRT1 and an LXR, including

- a) transfecting a cell with a vector harboring a sequence encoding an epitope-tagged first member of the specific binding pair;
- b) further transfecting the cell with a vector harboring a sequence encoding the second member of the specific binding pair;
- c) contacting the cell with the candidate substance;
- d) lysing the cells, contacting the cell lysates with an antibody specific for the epitope tag, and recovering immunoprecipitates comprising a complex of the SIRT1 and the LXR with an antibody-specific adsorbent;
- e) carrying out a Western blot procedure using antibodies specific for SIRT1 and an LXR;

whereby a difference in complex formation detected in the presence of the candidate compared with the absence of the candidate indicates that the candidate modulates the interaction of the SIRT1 with the LXR.

In these assay methods, transfection with vectors may be carried out by techniques such as those that have been described above, incorporated herein by reference. Any equivalent technique known to workers of skill in the field of the invention, not specifically described herein, may likewise be used to accomplish transfections.

Various of the assay methods further include transfection with a vector harboring a reporter gene operably driven by an LXRE. The reporter gene will be activated by a cellular component that specifically binds the LXRE to express the reporter gene. The reporter gene product is detectable in the experimental system devised to conduct the assay. In general, a

reporter gene encodes a protein, factor, or enzyme activity that upon expression is readily detectable by chemical or physical detection methods. Nonlimiting examples of reporter genes include genes encoding an enzyme such as horse radish peroxidase or firefly luciferase, a chromophoric protein or a fluorescent protein such as green fluorescent protein, and the like. Any equivalent reporter gene known to workers of skill in the field of the invention, not specifically described herein, may likewise be used to transfect the cell in order to carry out the assay for identifying potential active agents.

Various additional embodiments of the assay methods include transfecting a cell with a vector harboring a sequence encoding an epitope-tagged first member of the specific binding pair made up of SIRT1 and an LXR. Epitopes that are usable for this purpose include any epitope bound by a specific antibody. A nonlimiting example of an epitope is the FLAG epitope; others are widely known to workers of skill in the field of the invention. Any equivalent epitope, not specifically described herein, may be used to as a chimera with a member of the specific binding pair in order to carry out the assay for identifying potential active agents.

Assays for potential active agents are frequently carried out using high throughput screens in highly replicated parallel assay apparatuses. Multiwell plates may be employed in these systems such that a large number of assays are carried out simultaneously. The candidate compounds employed in assays for detecting a potential active agent are commonly obtained as the result of chemical synthesis of combinatorial libraries of chemical compounds, in which various moieties and/or substituents in a family of related compounds are systematically varied. Analogous chemical libraries are commonly already available in stocks of compounds accessible to a person or an entity. Further analogous chemical libraries include chemicals obtained from disparate natural sources, as well as derivatives of such natural compounds that have been further modified using the methods of combinatorial chemistry. In general, libraries of chemical compounds are widely known to workers of skill in the field of the invention. Any equivalent method for preparing a candidate compound, or for preparing a library of candidate compounds, known to workers of skill in the field of the invention, may be used in carrying out an assay to identify a potential active agent.

EXAMPLES

Methods

Plasmids

The plasmids pBabe-SIRT1 and pCMV-FLAG-HsSIRT1 have been described^{15, 16}.

Animals and plasma lipoprotein analysis

SIRT1^{+/+}, SIRT1^{+/-}, and SIRT1^{-/-} mice in mixed 129/sv-CD1 background were housed in a temperature-controlled (22 °C) facility with a daylight cycle from 7 a.m. to 7 p.m. and were given access to food (with 0.02% cholesterol) and water ad libitum.

Cells, retroviral infection, and transfection

The SIRT1^{+/+}, SIRT1^{+/-}, and SIRT1^{-/-} mouse embryonic fibroblasts (MEFs) were isolated from E14.5 embryos as previously described⁷³, and were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% FBS and antibiotics. Immortalized MEFs (MEFIs) were generated by transfecting primary MEFs with a pRS-SV40T plasmid. HEK293T and Phoenix cells (ATCC, Rockville, MD) were cultured in DMEM with 10% FBS and antibiotics. THP-1 cells (ATCC, Rockville, MD) were cultured in RPMI 1640 with 10% FBS and 0.5 mM β -mecaptoethanol.

All transfections were performed with lipofectamine 2000 plus reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, Ca). For retroviral infection, Phoenix cells were transfected with either pBabe or pBabe-SIRT1. Medium containing retroviruses was collected 48 h later, filtered, treated with polybrene (1mg/ml), and transferred on MEFs. Infected cells were then cultured with normal medium containing 1 μ g/ml puromycin.

Example 1. Plasma Cholesterol in Wild Type and SIRT1 Knockout Mice.

Ten wildtype, 13 SIRT1^{+/-}, and 13 littermate SIRT1^{-/-} male mice in each group were analyzed. Animals were fasted for four hours from the beginning of the daylight cycle, then blood was collected and plasma was obtained by K₃-EDTA treatment. Plasma total cholesterol, HDL, and LDL levels were measured by the enzymatic, colorimetric assay kits (Wako Diagnostics, Richmond, Va.). 100 μ l of pooled plasma from 4 SIRT1^{-/-} and 4 littermate SIRT1^{+/+} males were size fractionated using two fast-performance liquid chromatography (FPLC) columns (Superose 6B columns, Amersham-Pharmacia Biotech, Piscataway, NJ). A representative profile is shown from three independent experiments. Fractions from FPLC were then analyzed for cholesterol contents with the enzymatic, colorimetric assay kit from

Wako. The experiment was repeated three times with total of 12 *SIRT1*^{-/-} and 12 littermate *SIRT1*^{+/+} males. Results were compared with student's t-test.

Fig. 1a shows that *SIRT1*^{-/-} mice had slightly lower total plasma cholesterol levels, compared with wildtype age- and gender-matched mice. Upon inspection of Fig. 1, panels b (direct enzymatic assay) and d (size exclusion chromatographic/enzymatic assay), it is seen that this is primarily due to an approximately 40% reduction in the cholesterol carried in HDL (*, $p < 0.001$), the principal cholesterol-rich lipoprotein (80-90% of total) in murine plasma. The plasma LDL cholesterol levels were similar in *SIRT1*^{-/-} and control mice (Fig. 1c and d). As a result, the total cholesterol/HDL ratio of *SIRT1*^{-/-} mice was abnormally high (2.57 ± 0.98 vs. 1.36 ± 0.19 for wildtype controls, $p < 0.001$), whereas the HDL/LDL ratio was abnormally low (1.92 ± 0.94 vs. 3.56 ± 1.24 , $p < 0.001$). Because of the pleiotropic effects in the *SIRT1*^{-/-} mice, *SIRT1*^{+/-} heterozygotes, which are phenotypically similar to wildtype, were also examined. HDL levels were also found to be significantly reduced in *SIRT1*^{+/-} heterozygotes (Fig 1b, (*, $p < 0.001$)).

Total cholesterol (TC), free cholesterol (FC), cholesterol ester (CE), and phospholipids (PL) contents were measured in HDL fractions (fraction # 25-38) from the 12 pairs of animals, shown in Fig. 1, panel e. The relative reductions in *SIRT1*^{-/-} mice of the levels of total cholesterol, free cholesterol, cholesterol ester and phospholipids in plasma HDL were similar, about 25-30% (Fig. 1e; **, $p < 0.01$). The size distribution of HDL was normal (Fig. 1d) in *SIRT1*^{-/-} mice, suggesting that the reduction in HDL was a consequence of fewer particles rather than of particles with altered composition or structure.

Example 2. Cholesterol in Tissues of Wild Type and *SIRT1* Knockout Mice.

To determine the total cholesterol levels in mouse tissues, *SIRT1*^{-/-} mice and control littermates were fasted for four hours from the beginning of the daylight cycle before sacrificing. Tissues were then harvested and weighted. Total cholesterol from liver and testis were extracted and measured by GC as described previously^{70,71}. Total lipids including triglycerides were also dissolved into a solution containing 60% butanol, 13% methanol, and 27% Triton X-100, and measured with the enzymatic, colorimetric assay kits from Wako.

In *SIRT1*^{-/-} mice, abnormally low plasma HDL (Example 1) was associated with an increase in the accumulation of cholesterol within two tissues for which HDL is important as a source of exogenous cholesterol: the testis, which uses HDL cholesterol for sterol stores and steroidogenesis²⁵ and is the organ that has highest relative levels of *SIRT1* protein^{26,27},

and the liver, which plays a central role in cholesterol and HDL homeostasis²⁸ (Fig. 1f, n=8; ***, $p<0.01$).

The decrease in plasma HDL cholesterol and increase in accumulation of tissue cholesterol in the liver and testis in *SIRT1*^{-/-} mice could occur because of increases in lipoprotein cholesterol uptake or decreases in cellular cholesterol efflux (export) to lipoproteins. Increase in tissue cholesterol could also be due to increases in local cholesterol synthesis. However, we observed in the liver and testis essentially normal levels of mRNA for the highly sensitive, cholesterol-regulated gene encoding the rate controlling enzyme in cholesterol biosynthesis, HMG-CoA reductase (data not shown). Also, there were no apparent increases in these tissues in the protein levels of the major HDL receptor SR-BI (data not shown) that mediates cellular uptake of HDL cholesterol²⁹, nor increases in the rates of uptake of lipid from HDL in *SIRT1*^{-/-} mouse embryonic fibroblasts (MEFs; data not shown). Furthermore, there were substantial decreases, rather than increases, in the mRNA levels in testis and liver of two other major lipoprotein receptors, the LDL receptor (LDLR), and the VLDL/chylomicron receptor LRP (data not shown). Because neither increased *de novo* cholesterol synthesis nor receptor-mediated import appears to account for the increases in tissue and decreases in plasma cholesterol in *SIRT1*^{-/-} mice, it seemed likely that reductions in cellular cholesterol efflux might account for these observations.

Example 3. Efflux of Cholesterol from Wild Type and *SIRT1*^{-/-} Cells.

Reverse cholesterol transport is the process whereby excess cholesterol in peripheral tissues is transported to the liver for elimination from the body^{30,31}. The first step of this process is the efflux of cholesterol from cells to lipoproteins, particularly HDL. Several cell surface cholesterol transport proteins can mediate cholesterol efflux, including SR-BI³²⁻³⁵, ABCG1^{36,37}, and the best characterized of these, the ATP-binding cassette (ABC) transporter called ABCA1 that transfers unesterified cholesterol and phospholipids to lipid-poor apolipoproteins (mainly apoA-I) to form HDL particles^{30,38-44}. To test the effects of SIRT1 on this process, apoA-I-mediated cholesterol efflux was measured in two distinct cultured cell systems in which cells were labeled with [³H]cholesterol and the efflux of labeled cholesterol to apoA-I in the extracellular medium was monitored.

For MEFs, cholesterol efflux assay was performed as described⁷² with modifications. Primary *SIRT1*^{-/-} and littermates control *SIRT1*^{+/+} or *SIRT1*^{+/-} MEFs were plated in 12-well plates at 50% confluence and cultured for overnight. Cells were then transferred into RPMI

1640/0.2% bovine serum albumin (BSA) for 24 h and cholesterol loaded by incubation with 50 µg/ml of LDL and 1 µCi/ml of [³H]cholesterol (NEN Life Science Products, Boston, Ma.) for additional 24 h. Cells were washed with PBS, equilibrated for 4 h in RPMI 1640/0.2% BSA, and then incubated in RPMI 1640/0.2% BSA with or without 15 µg/ml apoAI for 24 h. The medium was then collected, and cells were lysed with 0.2 N NaOH. The radioactivity recovered in the medium and cell lysates were measured, and the apoAI-mediated cholesterol efflux was calculated as the percentage of the radioactivity recovered in the medium over the total radioactivity subtracting of the nonspecific efflux in apoAI-free medium. The cholesterol efflux assays were performed in duplicates with 7 pairs of *SIRT1*^{-/-} and control *SIRT1*^{+/+} or *SIRT1*^{+/-} MEFs from 3 litters.

Using primary MEFs, wildtype control cells and *SIRT1*^{-/-} cells loaded with [³H]cholesterol were incubated with RPMI 1640 medium with or without 15 µg/ml apoAI. [³H]cholesterol levels in the cells and culture medium were measured 24 h later and apoAI-mediated cholesterol efflux was calculated. Fig 1g (left panel) shows that efflux from primary control *SIRT1*^{+/+} MEFs (filled bar) was almost three-fold higher than from *SIRT1*^{-/-} MEFs (open bar) (n=7; ****, *p*<0.001).

For the human monocytic cell line THP-1 cells, cells were treated with 10 mM nicotinamide in RPMI1640/10% FBS for 48 h, then cholesterol loaded with 50 µg/ml of LDL and 1 µCi/ml of [³H]cholesterol in RPMI1640/1% FBS/0.2% BSA medium for 24 h. Cells were washed with PBS and incubated in RPMI 1640/0.2% BSA with or without 15 µg/ml apoAI for 24 h. The medium and cells were then separated, and cells were lysed with 0.2N NaOH. The radioactivity recovered in the medium and cell lysates were measured and the apoAI-mediated cholesterol efflux was calculated as described above.

Treatment of THP-1 with the SIRT1 inhibitor nicotinamide (Nico)⁴⁵ also suppressed [³H]cholesterol efflux by roughly three-fold (Fig. 1g, right panel). The experiment was performed in triplicate and repeated twice (*p*<0.01). Thus loss of SIRT1 in MEFs and inhibition of SIRT1 activity in monocytes reduces apoAI-mediated cholesterol efflux. These results support the possibility that reductions in cholesterol efflux might lead to reduced plasma HDL cholesterol and increased tissue cholesterol in *SIRT1*^{-/-} mice.

Example 4. Levels of ABCA1 mRNA in Wild Type and SIRT1 Knockout Tissues.

To examine whether lower cholesterol efflux could produce reduced plasma HDL cholesterol and increased tissue cholesterol in *SIRT1*^{-/-} mice, the levels of ABCA1 mRNA in liver, testis, and ovary were compared.

Total RNA was isolated from mouse livers, testes, thioglycolate-stimulated peritoneal macrophages, and ovaries by a Qiagen RNeasy mini-kit (Qiagen Inc., Calencia, Ca.). For real-time PCR analysis, cDNA was synthesized by SuperScript III reverse transcriptase (Invitrogen, Carlsbad, Ca) with random primers. The resulting cDNA was then subjected to PCR analysis with gene-specific primers in the presence of Cybergreen (Qiagen Inc., Calencia, Ca.). Relative abundance of mRNA was obtained by normalization to cyclophilin levels. For northern hybridization, RNA (10 µg/lane) was separated on formaldehyde-agarose gels (1.0%), transferred to GeneScreen Plus membranes (NEN Life Science Products, Boston, Ma.), and hybridized according to standard procedures. The mRNA levels of ABCA1 were quantified by phosphoimager and normalized against the corresponding actin levels.

ABCA1 mRNA expression was reduced by 40-50% in *SIRT1*^{-/-} mice in the three tissues tested, compared with wild type (Fig. 2a). Consistent with the idea that this reduction might have contributed to the reduced levels of plasma HDL, in *ABCA1*^{+/-} heterozygous mice, the HDL cholesterol is reduced to ~60% of *ABCA1*^{+/+} controls⁴⁶, similar to the reduction in *SIRT1*^{-/-} mice.

Example 5. Activity of the ABCA1 Promoter.

Transcription of ABCA1 is induced by increasing cellular cholesterol, and the LXR nuclear receptor transcriptional factors mediate this response⁴⁷⁻⁴⁹. LXR/retinoid X receptor (RXR) heterodimers are activated by oxysterols⁵⁰ and play key roles in the regulation of whole body cholesterol homeostasis, lipid biosynthesis, inflammatory response, carbohydrate metabolism, and obesity⁵¹. To determine if the effects of SIRT1 on expression of ABCA1 are mediated by LXRs, reporter constructs were generated in which expression of the firefly luciferase gene was driven by either the mouse ABCA1 promoter or a control mutant promoter in which an eight-nucleotide deletion removed the LXR response element (LXRE). Previous studies have shown that a combination of 22(R)-hydroxycholesterol (22(R)-HC, a LXR agonist) and 9-cis retinoic acid (9-cisRA, a RXR agonist) dramatically stimulates the activity of the LXRE in the ABCA1 promoter⁴⁷.

The luciferase reporter plasmid pGL3-ABCA1 was created by inserting the – 847 to +244 of mouse ABCA1 promoter region into KpnI-XhoI sites of pGL3 vector (Promega, Madison, Wi). pGL3-ABCA1-LXRE, the LXRE mutant version of pGL3-ABCA1, was created by deleting the LXRE from wildtype promoter of mouse ABCA1.

For the luciferase assay, MEFs or HEK293T cells plated in 24-well plates were transfected with 100 ng of pGL3-ABCA1 or pGL3-ABCA1-LXRE, and 10 ng of pRL-TK (*Renilla* luciferase; Promega). The cells transfected with wildtype (WT) or LXRE mutant (– LXRE) mouse ABCA1 promoter driven luciferase reporter vectors were treated with 1 μ M Trichostatin A (TSA), an inhibitor of class I and II deacetylases, or 10 mM nicotinamide (Nico), or both 10 mM nicotinamide + 1 μ M TSA. Cells transfected with the LXRE mutant vector were incubated in DMEM with 10% FBS in the absence (solid bars) or presence (open bars) of 10 μ M 22(R)-HC and 1 μ M 9-cisRA (Fig. 2b). Luciferase activity was then measured by using the Dual-Luciferase Reporter Assay System (Promega) 6 or 24 h later. The final GL (firefly luciferase) activity was normalized with co-expressed RL (*Renilla* luciferase) activity. The experiments were performed in duplicate and were repeated 3 times.

The wildtype and mutant reporter constructs were expressed in HEK293T cells (Fig. 2b, WT (left panel) or –LXRE (right panel)). Luciferase expression from the wildtype was stimulated 8-fold by treatment with 22(R)-HC+9-cisRA (Fig. 2b, left panel). Moreover, this induced expression was inhibited 50% by the inhibitor nicotinamide which inhibits class III deacetylases such as SIRT1 (a), but was not inhibited by TSA. Constructs missing the LXRE were unresponsive to the LXR agonist and showed a low basal activity (Fig. 2b, right panel). Together, these findings suggest that SIRT1, but not other classes of deacetylases, modulates LXR activity at the ABCA1 promoter, interestingly as a positive regulator.

In addition, it is seen that the system used in these experiments affords a method for assessing the modulation of LXR-dependent processes by a candidate substance.

Example 6. Effect of SIRT1 Activity on the ABCA1 Promoter.

To directly determine the influence of SIRT1 on the ABCA1 promoter, these promoter reporter constructs were introduced into immortalized *SIRT1*^{+/+} and *SIRT1*^{–/–} MEFs that were or were not infected with the pBabe-mT1 vector to express SIRT1 protein (Fig. 2c). *SIRT1*^{+/+} and *SIRT1*^{–/–} MEFs were generated and infected with pBabe-SIRT1 (+) or a pBabe control vector (–) as described in Example 3.

The insert in Fig. 2c, right panel, shows the immuno-blotting results of the expression levels of SIRT1 in these four cell lines; tubulin was used as loading control. Expression of SIRT1 from this vector was slightly lower than that of endogenous SIRT1 in *SIRT1*^{+/+} MEFs.

MEFs were then transfected with wildtype (WT) or LXRE mutant (-LXRE) mouse ABCA1 promoter driven luciferase reporter vectors in the absence (filled bar) or presence (open bar) of 10 μ M 22(R)-HC and 1 μ M 9-cisRA. Luciferase activities were measured after 24 h. All cells with the wildtype promoter exhibited marked stimulation of luciferase expression when treated with the LXR/RXR activators (Fig.2 c, left panel), whereas the low basal activity in cells with the control mutant (-LXRE) reporter was not stimulated by these activators (Fig. 2c, right panel). Most importantly, LXRE-dependent activity of the wildtype promoter construct was 40% lower ($p < 0.02$) in cells that were SIRT1 deficient (*SIRT1*^{-/-} MEFs) than in cells that were *SIRT1*^{+/+}, or were reconstituted with SIRT1 (pBabe-T1). These findings support the model that SIRT1 stimulates the ABCA1 promoter via LXR/RXR acting at the LXRE.

The experimental system in this Example affords a method for assessing the modulatory effect of a candidate substance on the SIRT1-dependent effect of LXRs.

Example 7. Interaction of SIRT1 with the LXRE of ABCA1 Promoter.

To determine if SIRT1 binds directly to the LXRE in the ABCA1 promoter, we used the chromatin immunoprecipitation (ChIP) assay and probed with different primers for the murine ABCA1 promoter.

ChIP analysis was performed as described⁷⁶ with modifications. Briefly, cells were crosslinked in 1% paraformaldehyde in cultured media for 15 min at room temperature and crosslinking was terminated with 0.125M glycine. Cells were then harvested in HEPES buffer (50 mM HEPES-NaOH, pH 7.9, 140 mM NaCl, 1 mM EDTA, 10 % Glycerol, 0.5% NP40, 0.25% Triton X-100, and Complete™ protease inhibitor mixture (Roche, Indianapolis, In)), and nucleus were purified. Purified nucleus were resuspended in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, and Complete™ protease inhibitor mixture), and sonicated on ice to an average length of 1 kb. Sonicated supernatants were diluted into IP buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 10 % Glycerol, 1% Triton X-100, 0.1% Na-Deoxycholate, and Complete™ protease inhibitor mixture), precleaned twice with protein A beads. Precleaned chromatin samples were then incubated with anti-SIRT1 rabbit polyclonal antibodies (Upstate, Charlottesville, Va) overnight at 4 °C, and immune complexes were

recovered by adding protein A beads for 2 h. Recovered immunoprecipitates were then washed with IP buffer twice, IP buffer with 500 mM NaCl twice, and RIPA buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 250 mM NaCl, 0.5% NP40, 0.5% Na-deoxycholate, and Complete™ protease inhibitor mixture) twice. Chromatins in immunoprecipitates were then eluted with 10×TE/1% SDS at 65 °C for 15 min. Eluted chromatins were incubated at 65 °C for at least 6 h to reverse crosslinking, treated with proteinase K and RNaseA, and extracted with phenol/chloroform and chloroform. Final DNA fragments were recovered by LiCl/glycogen/ethanol precipitation and pellets were resuspended in water and assayed by PCR.

For the LXRE of mouse ABCA1 promoter, PCR reaction was performed using primers

5'-GCTTTCTGCTGAGTGACTGAACTAC-3' (SEQ ID NO:13) :and
5'-GAATTACTGCTTTTTGCCGCG-3' (SEQ ID NO:14).

As the negative control of above ChIP assay, a fragment 6.2 kb upstream of LXRE on ABCA1 promoter was also amplified with primers

5'-GCAGCCCAACTCTTCAGAAC-3' (SEQ ID NO:15) and
5'-TCCCCTTTGTCTTTGTGGAC-3' (SEQ ID NO:16).

For the LXRE of human promoter, PCR reaction was performed using primers

5'-GCTTTCTGCTGAGTGACTGAACTAC-3' (SEQ ID NO:17) and
5'-TGCGTCTCTTTCTCCTACCC-3' (SEQ ID NO:18).

A fragment 7.5 kb upstream of LXRE was amplified with primers

5'-AGGCAGGTGGATCATTTGAG-3' (SEQ ID NO:19) and
5'-CCAAACATCTGGGCTTCTGT-3' (SEQ ID NO:20)

as a negative control. Experiment was performed at least three times, and representative data are shown.

Fig.2d top shows results of probing with LXRE- or upstream-specific PCR probes before (Input) or after (αSIRT1) immunoprecipitation with antibodies to SIRT1. It is seen that in the experimental treatment (αSIRT1) antibody treatment precipitated fragments of chromatin containing the LXRE from *SIRT1*^{+/+} MEFs (left lane, upper image showing a dark gray band), but not *SIRT1*^{-/-} MEF controls (right lane, upper image showing solid black), and did not precipitate fragments containing DNA 6.2 kb upstream of the LXRE from either cell line (lower image, both lanes, showing solid black). Similar results were found in human HEK293T (Fig 2d, bottom) and THP-1 (data not shown) cells transfected with a vector to

express FLAG epitope tagged human SIRT1 or a negative control vector, and precipitated with anti-FLAG antibody.. Thus, SIRT1 binds to the ABCA1 promoter in close proximity to the LXRE.

Example 8. Interaction of SIRT1 with LXR α and LXR β

SIRT1 does not directly bind to DNA ²⁶, so its presence on the ABCA1 promoter requires binding to proteins associated with DNA, such as the LXRs. There are two members of the LXR transcriptional factor family, LXR α and LXR β ^{50, 52}. The expression of LXR α is tissue-specific, whereas that of LXR β is ubiquitous. To determine if there were association between SIRT1 and LXRs by co-immunoprecipitation, murine LXR α and LXR β cDNAs were cloned into HA-tagged expression vectors. The SIRT1 expression vector pBabe-SIRT1 was then transfected into HEK293T cells with or without the vectors for the HA-tagged LXRs.

In this Example, the possible interaction between SIRT1 and LXR α or LXR β was examined. Plasmids expressing NH₂-terminal HA-tagged LXR α (HA-LXR α) and LXR β (HA- LXR β) were created by cloning the full-length mouse LXR α or LXR β cDNA downstream of (in-frame with) a HA epitope in a modified pcDNA3-NHA vector. HEK293T cells over-expressing mouse SIRT1 (MmSIRT1) and HA-LXR α , or MmSIRT1 and HA-LXR β , were harvested in NP40 buffer (10 mM Tris-Cl, pH 8.0, 150 mM NaCl, 0.5% NP40, and Complete™ protease inhibitor mixture) and lysed by passing through a 18-gauge syringe needle 10 times. After rocking at 4 °C for 1 h, lysates were subjected to centrifugation at 16,000 × g for 15 min. The resulting supernatant was then diluted into NP40 buffer with 1% BSA and immunoprecipitation was initiated by adding 3 µg of anti-HA monoclonal antibodies (Santa Cruz Biotechnology). The mixture was then incubated with rocking at 4 °C for 2 h to overnight. Immune complexes were recovered by adding protein A beads for additional 1 h and immunoprecipitates were washed five times with 1 ml NP40 buffer. The total lysates and corresponding immuno-precipitation samples were then separated by SDS-PAGE and immuno-blotted with anti-SIRT1 polyclonal antibodies and anti-myc or anti-HA antibodies.

The monoclonal mouse anti-LXR α antibodies were from R&D system and the polyclonal goat anti-LXR α/β antibodies were from Santa Cruz Biotechnology. Human HEK293T cells expressing indicated proteins were lysed and immuno-precipitated (IP) with

anti-HA monoclonal antibodies as described above (Fig. 2 e). The total lysates (lanes 1-4) and anti-HA immuno-precipitation samples (lanes 5-8) were then separated by SDS-PAGE and probed with anti-SIRT1 polyclonal antibodies (top images) and with anti-HA-LXR α antibodies (top panel) or anti-HA-LXR β antibodies (bottom panel). As shown in Fig. 2e, transfected murine SIRT1 protein (mT1) was cross-precipitated by anti-HA antibodies only when the tagged LXR α or LXR β was co-expressed in the cells (lanes 8, top and bottom panels). HA-LXR also co-precipitated the endogenous human SIRT1 protein (hT1), which exhibited a slightly lower mobility in the gels. Thus, direct or indirect binding of SIRT1 to LXR is probably responsible for the association of SIRT1 with the LXRE of the ABCA1 promoter in intact cells.

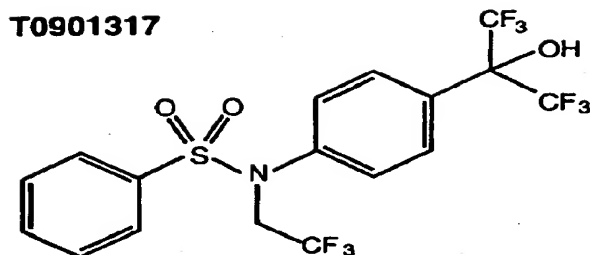
Furthermore, the immunoprecipitation experiment described in this Example affords a sensitive assay for ability of a test substance to modulate the interaction of SIRT1 with either LXR α or LXR β .

Example 9. LXR target mRNAs in livers of *SIRT1*^{-/-} mice.

The interaction between LXRs and SIRT1 raises the possibility that loss of function of SIRT1 could impair LXR-mediated lipid homeostasis in response to dietary cholesterol. To test this hypothesis, the mRNA levels of an array of LXR target genes, including ABCA1, were analyzed in the livers of *SIRT1*^{-/-} mice (Fig. 3a), using total RNA from livers of wildtype control animals (filled bars) and *SIRT1*^{-/-} animals (open bars). The analyses were done by real-time PCR. Even though the levels of LXR mRNA were normal in *SIRT1*^{-/-} liver, many LXR targets, such as SREBP1, ABCA1, ABCG1, ABCG5, and LDLR, were decreased compared to the *SIRT1*^{+/+} controls (n=3, *, *P*<0.05). Levels of mRNA of control genes known not to be LXR targets^{53, 54} were not significantly altered in *SIRT1*^{-/-} mice.

Example 10. Relative levels of metabolites in livers of mice treated with the LXR agonist T0901317.

To gain further insight into the effect of SIRT1 on LXR function *in vivo*, wildtype and *SIRT1*^{-/-} animals were analyzed for their response to 8-day oral administration of the LXR agonist T0901317 (N-(2,2,2-trifluoro-ethyl)-N-[4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl-ethyl)-phenyl]-benzenesulfonamide)⁵³.



To detect ABCA1 proteins in mouse tissues, *SIRT1*^{+/+} or *SIRT1*^{-/-} livers were homogenized in RIPA buffer. Liver extracts were then mixed with SDS sample buffer and denatured at 37 °C for 20 min to prevent the aggregation of ABCA1 at high temperature. Samples were then resolved in 7.5% SDS-PAGE and blotted with anti-ABCA1 polyclonal antibodies (R80 antibodies, gift from Drs. M. Fitzgerald and M. Freeman).

2-6 month old *SIRT1*^{-/-} and control males were fed with T0901317 (10mg/kg) by oral gavage, plasma were collected before and after feeding for plasma lipid analyses. Tissues were collected after feeding for further analyses. Total RNA from liver of wildtype animals without (black filled bar) or fed with 10mg/kg T0901317 (black striped bar) and *SIRT1*^{-/-} mice without (open bar) or with T0901317 (gray striped bar) were analyzed by real-time PCR. T0901317 feeding of wildtype animals significantly induced the expression of several LXR targets: SREBP-1c, LPL, and ABCA1 at the RNA level (Fig 3b) (n=4, **, *p*<0.05). However, the induction of these targets in *SIRT1*^{-/-} mice was blunted. Total protein lysates were obtained from livers of indicated mice and analyzed for the levels of ABCA1 protein by immunoblotting (Fig 3c). (As previously observed, ABCA1 proteins comprise multiple bands by western blotting⁷⁸.)

Plasma results in Fig. 3, panels d, e, and f represent the same mice before and after 8-days administration of T0901317. Liver results in Fig. 3, panel d compare littermates without or with T0901317, as in Fig. 3, panels b (Example 9) and c (this Example) above. Triglycerides from plasma (Fig. 3 d, left panel) and liver (Fig. 3 d, right panel) of wildtype mice without (black filled bar) or with T0901317 (left striped bar) and *SIRT1*^{-/-} animals without (open bar) or with T0901317 (right striped bar) were analyzed. A reduced response in *SIRT1*^{-/-} animals to LXR agonist T0901317 induction⁵³ of plasma and hepatic triglyceride accumulation was observed (Fig. 3d; n=5, ***, *p*<0.001). Lower plasma cholesterol levels in treated mice were also seen (Fig 3e; n=5, ****, *p*<0.02) especially in HDL size particles as

determined by cholesterol assays of FPLC lipoprotein fractions (Fig. 3f, n=5)). Because T0901317 results in elevation of plasma and hepatic triglycerides through induction of SREBP-1c⁵³, the low levels of SREBP1-c mRNA in *SIRT1*^{-/-} mice (Fig. 3a) may explain the reduction of triglycerides induced by T0901317. Thus, not only was the effect of SIRT1 on the ABCA1 promoter in reporter assays dependent on LXR, the functions of LXR and its agonist in vivo was also dependent on SIRT1.

Example 11. Effect of SIRT1 on LXR Protein Expression.

SIRT1 directly interacts with and deacetylates many target proteins, such as histones 12, p53 15-17, FOXO transcriptional factors 18, 19, and PGC-1 α 23, 24. Deacetylation of these targets by SIRT1 either represses (p53 and FOXO at some promoters) or activates (PGC-1 α and FOXO at other promoters) their transcriptional activities. To investigate how SIRT1 positively regulates LXR, the levels of endogenous LXR protein in the livers of *SIRT1*^{+/+} and *SIRT1*^{-/-} mice were first determined. Surprisingly, LXR protein levels were dramatically increased in *SIRT1*^{-/-} animals (Fig. 4a), even though the ability of LXR to activate targets was reduced compared to wildtype (Example 9; Fig. 3a).

HEK293T cells expressing HA-LXR β driven by an exogenous CMV promoter were transfected either with pSuper vector, or with a pSuper-SIRT1 RNAi construct. Cells were harvested 3 days after transfection and the levels of HA-LXR β and SIRT1 were analyzed by western blotting using antibodies to HA and SIRT1. It was found that both RNAi-mediated knockdown of SIRT1 expression (Fig. 4b, lanes 3 and 4). and inhibition of SIRT1 activity by nicotinamide – but not inhibition of other deacetylases by TSA – (Fig. 4c) resulted in elevated levels of HA-LXR β protein. Since there was no detectable reduction of LXR mRNA in *SIRT1*^{-/-} mice (Fig. 3a), SIRT1 regulates LXR protein levels post-transcriptionally.

HEK293T cells expressing HA-LXR β were treated with either control medium (Fig. 3c, lane 1), or medium with 1 μ M TSA (lane 2), 10 mM nicotinamide (lane 3), 25 μ M MG132 (carbobenzoyloxy-Leu-Leu-Leu-al, a proteasome inhibitor) (lane 4), or 10 mM nicotinamide plus 25 μ M MG132 (lane 5) for 6 h. Cells were then harvested and the levels of HA-LXR β and SIRT1 were analyzed by western blotting using antibodies to HA (upper images) and SIRT1 (middle images). Inhibition of SIRT1 activity by nicotinamide (lane 3) – but not inhibition of other deacetylases by TSA (lane 2) – resulted in elevated levels of HA-LXR β protein (upper images). Since there was no detectable reduction of LXR's mRNA in *SIRT1*^{-/-} mice (see Example 9, Fig. 3a), SIRT1 regulates LXR protein levels post-transcriptionally. It

is believed that inhibition of SIRT1 activity by nicotinamide increases the protein levels of LXR by interfering with proteasome-mediated LXR degradation.

The basis for the finding that LXR activity is inversely correlated with protein levels was next examined. Since acetylation of lysine residues can block ubiquitination and SIRT1 is a deacetylase, loss of SIRT1 activity might stabilize LXR proteins by increasing the steady state level of LXR acetylation, thus inhibiting the ubiquitin/proteasome degradation pathway. If this were true, inhibition of proteasome activity by MG132 should increase the steady state levels of LXR when SIRT1 is active, but not when SIRT1 is inhibited by nicotinamide. This in fact was observed in HEK293T cells expressing HA-tagged LXR β (Fig. 4c, lane 4 vs. lane 5).

HEK293T cells were co-transfected with constructs expressing an empty pBabe vector, HA-LXR β , pBabe-SIRT1, and pBapbe-hSIRT1HY (an inactive deacetylase SIRT1 mutant (see Vaziri et al., 2001)). 40 h later, cells were incubated with 25 μ M MG132 for 1 h and harvested. HA-LXR β was then immunoprecipitated using antibodies to HA followed by western blotting using antibodies to ubiquitin, HA and SIRT1. The results show that expression of an active SIRT1 transgene (hSIRT1) but not the inactive mutant hSIRT1HY increased the amount of ubiquitin-conjugated LXR in transfected HEK293T cells (Fig. 4d, lane 3 vs. lane 4).

Example 12. Acetylation of LXR in vivo

To detect the acetylation of LXR and deacetylation of LXR by SIRT1 in vivo, HEK293T cells co-transfected with HA-LXR β , and with either pBabe, pBabe-HsSIRT1, or pBabe-HsSIRT1H355Y, were cultured in medium containing sodium [3H]acetate as described ⁷⁷ with modification. Briefly, 40 h after transfection, 2 mCi/ml sodium [3H]acetate, the proteasomal inhibitor MG132 (25 μ M), 22(R)-HC (10 μ M), and 9-cis-RA (1 μ M) were added to the culture for 1 h. Cells were then washed twice with cold phosphate-buffered saline and lysed in NP40 buffer. The lysate was centrifuged at 16,000 \times g for 15 min at 4 $^{\circ}$ C. HA-LXR β was then immunoprecipitated with anti-HA antibody and separated by SDS-PAGE. Gels containing [3H]acetate-labeled HA-LXR β were first stained with Coomassie Brilliant Blue and then enhanced by impregnating with a commercial fluorography enhancing solution (Amplify, Amersham Biosciences) for 30 min. Dried gels were subjected to autoradiography at -70 $^{\circ}$ C for 3-7 days. The same samples were also immuno-blotted with anti-SIRT1 antibodies.

The results are shown in Fig. 4e. HA-LXR β was acetylated under normal culture conditions (lane 1). Furthermore, activation of LXR β by treatment with 22(R)-HC + 9-cisRA stimulated deacetylation (and/or possibly inhibited acetylation; lane 2). It appears likely that SIRT1 is responsible, at least in part, for the deacetylation of LXR, because, as shown in Fig. 4f, the levels of [3H] acetate-labeled HA-LXR were substantially lower in cells expressing hSIRT1 (pBabe-hSIRT1; lane 2), than in control cells transfected with an empty vector (pBabe; lane 1) or one encoding an enzymatically inactive SIRT1 (pBabe-hSIRT1HY; lane 3). These findings suggest that deacetylation of LXR β by SIRT1 both facilitates its turnover and increases its activity.

Example 13. Effect of inhibition of SIRT1 or proteasome-mediated LXR degradation on the transcription activity of LXR on the ABCA1 promoter.

HEK293T cells transfected with wildtype (WT; Fig. 4g, left panel) or LXRE mutant (-LXRE; Fig. 4g, right panel) mouse ABCA1 promoter driven luciferase reporter vectors were treated with either 10 mM nicotinamide (Nico), 25 μ M MG132, or both 10 mM nicotinamide + 25 μ M MG132, in the absence (solid bars) or presence (open bars) of 10 μ M 22(R)-HC and 1 μ M 9-cisRA for 6 h. Luciferase activities were measured and normalized as in Example 5.

It was found that nicotinamide blunted the induction of the luciferase reporter driven by the ABCA1 promoter significantly (Fig. 4g, left panel). Moreover, the proteasome inhibitor MG132 inhibited the reporter to the same extent as nicotinamide, and the combination of nicotinamide and MG132 did not result in a further inhibition. The control reporter missing the LXRE displayed basal activity that was not altered by nicotinamide or MG132 (Fig. 4g, right panel). These findings suggest that the deacetylation of LXR β by SIRT1 and its subsequent ubiquitination and degradation by the proteasome help activate the ABCA1 promoter, as discussed below.

These Examples show that SIRT1 positively regulates LXR transcription factors, and thus plays an important role in the regulation of cholesterol homeostasis. LXR and SIRT1 activate transcription of gene encoding the ABCA1 transporter, which mediates reverse cholesterol transport and HDL synthesis. These findings may be directly relevant to human medicine, as genetic and epidemiological studies indicate plasma HDL levels are inversely associated with risk of cardiovascular diseases³⁰ and possibly Alzheimer's disease^{5,6}. HDL

mediated reverse cholesterol transport may protect against atherosclerosis by clearing excess cholesterol from arterial cells^{55, 56} and protect against Alzheimer's disease by decreasing cholesterol-rich raft formation in the brain^{57, 58}. By activating LXR, SIRT1 may decrease the risk of ageing associated atherosclerosis and possibly Alzheimer's disease.

The physical and functional interactions between SIRT1 and LXRs suggest that SIRT1 exerts at least some of its effects *in vivo* by modulating LXRs. In fact, SIRT1-deficient mice share many metabolic defects with LXR deficient mice^{53, 54, 63}, such as decreased HDL cholesterol (Fig. 1) and triglyceride levels (Fig. 3d). Also, PEPCK, the rate-limiting enzyme in gluconeogenesis that can be repressed by a liver LXR agonist⁶⁴, is induced in SIRT1^{-/-} mice¹⁹. LXR dependent gene expression and energy homeostasis have recently been shown to be important for the innate immune response against microbes and mice lacking LXRs are highly susceptible to infection with bacteria⁶⁵. Therefore, it is possible that the observed susceptibility of SIRT1 knockout animals to lung infection²⁶ and eye infection (unpublished observation) is due to a blunted LXR activity in these animals.

The present findings suggest that SIRT1 promotes LXR activity by deacetylating it and causing its turnover (Fig. 5). Regulation of LXR by SIRT1 places this sirtuin at the center of cholesterol homeostasis pathways

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CLAIMS

We claim

1. An isolated complex comprising a mammalian SIRT1 protein and a mammalian LXR protein.
2. The complex described in claim 1 further comprising an LXR response element.
3. A method of forming a complex comprising a mammalian SIRT1 protein and a mammalian LXR protein, the method comprising combining a first composition comprising a mammalian SIRT1 protein, a second composition comprising a mammalian LXR protein and a third composition comprising a fragment of a cellular nucleic acid that includes a LXR response element.
4. A method of identifying an agent that modulates formation of a complex comprising a mammalian SIRT1 protein and a mammalian LXR protein, the method comprising
 - a) combining a first composition comprising a mammalian SIRT1 protein, a second composition comprising a mammalian LXR protein and a third composition comprising a fragment of a cellular nucleic acid that includes a LXR response element to provide a complex composition;
 - b) further either
 - i) contacting the first composition, the second composition, or the third composition with a fourth composition comprising the agent prior to the combining step, or
 - ii) contacting the complex composition with the fourth composition comprising the agent after the combining step; thereby providing a test composition, and
 - c) determining whether formation of the complex is modulated by the agent.
5. The method described in claim 4 wherein the agent increases formation of the complex.
6. The method described in claim 4 wherein the determining further comprises
 - d) combining the first composition that includes a mammalian SIRT1 protein, the second composition that includes a mammalian LXR protein, and the third composition comprising a fragment of a cellular nucleic acid that includes a LXR response element to provide a control composition not including the agent, and

- e) determining whether formation of the complex in the test composition is modulated in comparison with formation of the complex in the control composition.
7. Use of an agent that stimulates SIRT1 activity in the manufacture of a medicament for increasing the ratio of cholesterol bound to high density lipoprotein (HDL) to total cholesterol in the plasma of a mammal by administering the agent to the mammal.
 8. The use described in claim 7 wherein the mammal is a human.
 9. The use described in claim 7 wherein the agent comprises T0901317.
 10. Use of an agent that promotes formation of a complex comprising a mammalian SIRT1 protein and a mammalian LXR protein in the manufacture of a medicament for increasing the ratio of cholesterol bound to high density lipoprotein (HDL) to total cholesterol in the plasma of a mammal by administering the agent to the mammal.
 11. The use described in claim 10 wherein the mammal is a human.
 12. The use described in claim 10 wherein the agent comprises 22(R)-hydroxycholesterol or 9-cis retinoic acid, or both.
 13. A method of promoting ABCA1-mediated cholesterol efflux from a mammalian cell comprising introducing into the cell a nucleic acid that comprises a sequence encoding a protein deacetylase.
 14. The method described in claim 13 wherein the mammalian cell is a human cell.
 15. The method described in claim 13 wherein the protein deacetylase is a eukaryotic Sir2.
 16. The method described in claim 13 wherein the protein deacetylase is a mammalian SIRT1.
 17. The method described in claim 13 wherein the cell is further contacted with an agent that stimulates SIRT1 activity.
 18. The method described in claim 17 wherein the agent comprises T0901317.
 19. The method described in claim 13 wherein the cell is further contacted with an agent that promotes formation of a complex comprising a mammalian SIRT1 protein and a mammalian LXR protein.
 20. The method described in claim 19 wherein the agent comprises 22(R)-hydroxycholesterol or 9-cis retinoic acid, or both.

21. A method for assessing whether a candidate substance modulates an LXR-dependent process, comprising

- a) transfecting a cell with a vector harboring a reporter gene operably driven by an LXRE;**
- b) contacting the cell with the candidate; and**
- c) determining whether the candidate modulates the expression of the reporter gene in comparison with a cell not contacted with the candidate;**

whereby a difference in extent of occurrence of the LXR-dependent process detected between the presence and absence of the candidate indicates that the candidate modulates the LXR-dependent process.

22. A method for assessing whether a candidate substance modulates an SIRT1-dependent effect of an LXR, comprising

- a) transfecting a cell with a vector harboring an SIRT1 gene;**
- b) further transfecting the cell with a vector harboring a reporter gene operably driven by an LXRE promoter;**
- c) contacting the cell with the candidate; and**
- d) determining whether the candidate modulates the expression of the reporter gene in comparison with a cell not contacted with the candidate;**

whereby a difference in the extent of the SIRT1-dependent effect of the LXR detected between the presence and absence of the candidate indicates that the candidate modulates the SIRT1-dependent effect of an LXR.

23. A method for assessing whether a candidate substance modulates the formation of a specific binding pair comprising specific binding pair members SIRT1 and an LXR, comprising

- a) transfecting a cell with a vector harboring a sequence encoding an epitope-tagged first member of the specific binding pair;**
- b) further transfecting the cell with a vector harboring a sequence encoding the second member of the specific binding pair;**
- c) contacting the cell with the candidate substance;**
- d) lysing the cells, contacting the cell lysates with an antibody specific for the epitope tag, and recovering immunoprecipitates comprising a complex of the SIRT1 and the LXR with an antibody-specific adsorbent;**

e) carrying out a Western blot procedure using antibodies specific for SIRT1 and an LXR;

whereby a difference in complex formation detected in the presence of the candidate compared with the absence of the candidate indicates that the candidate modulates the interaction of the SIRT1 with the LXR.

Fig. 1

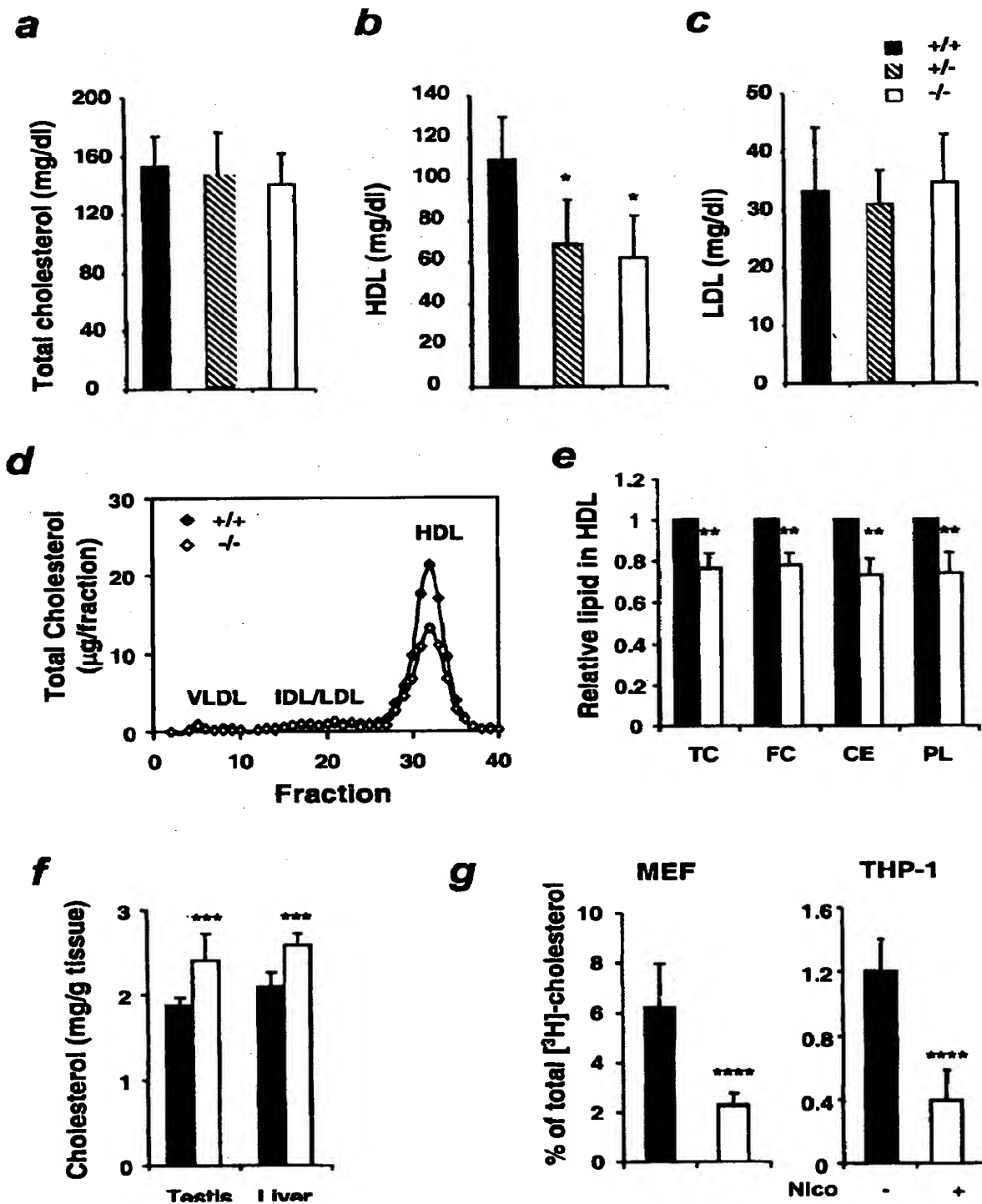


Fig. 2

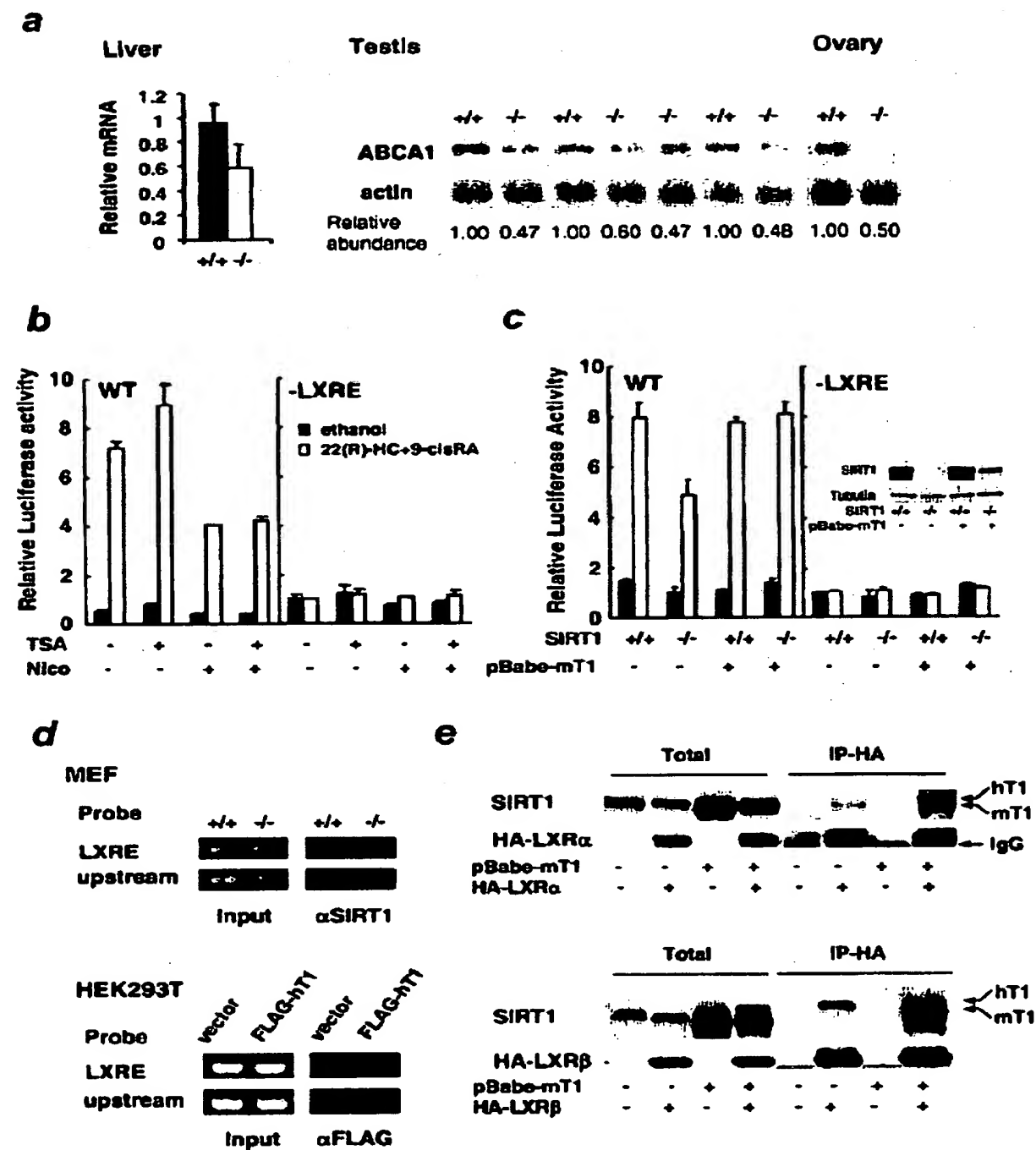


Fig. 3

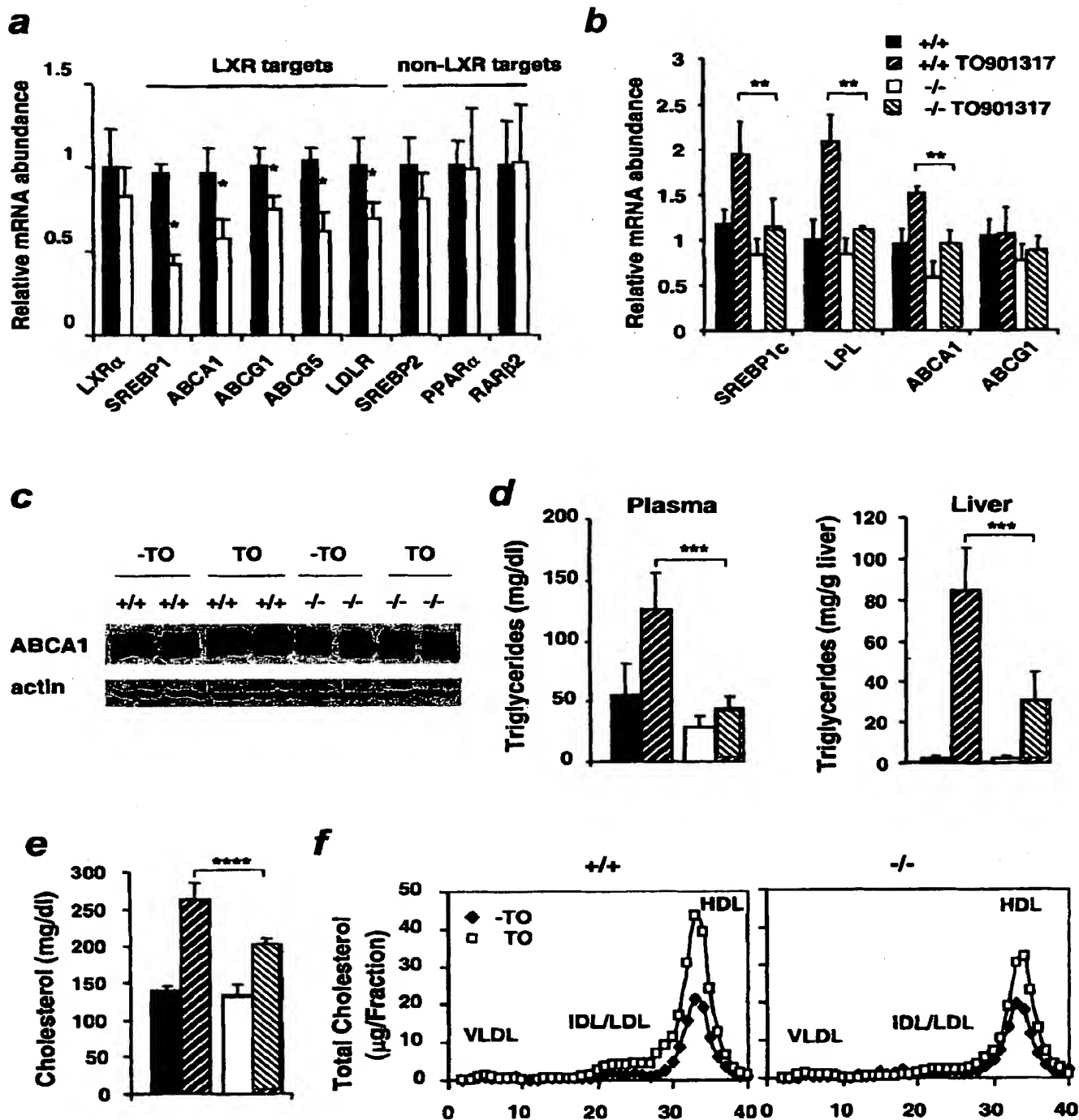


Fig. 4

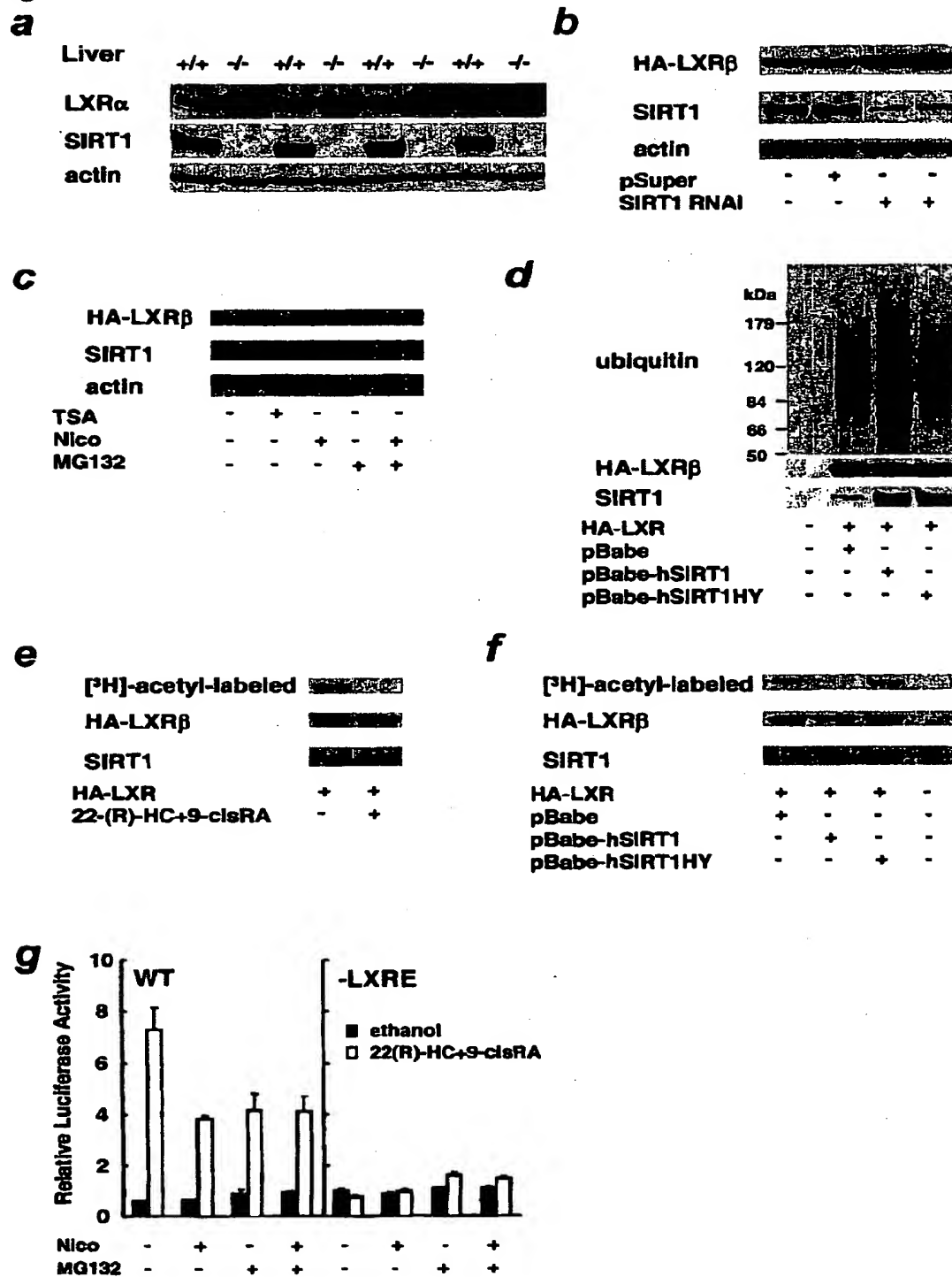
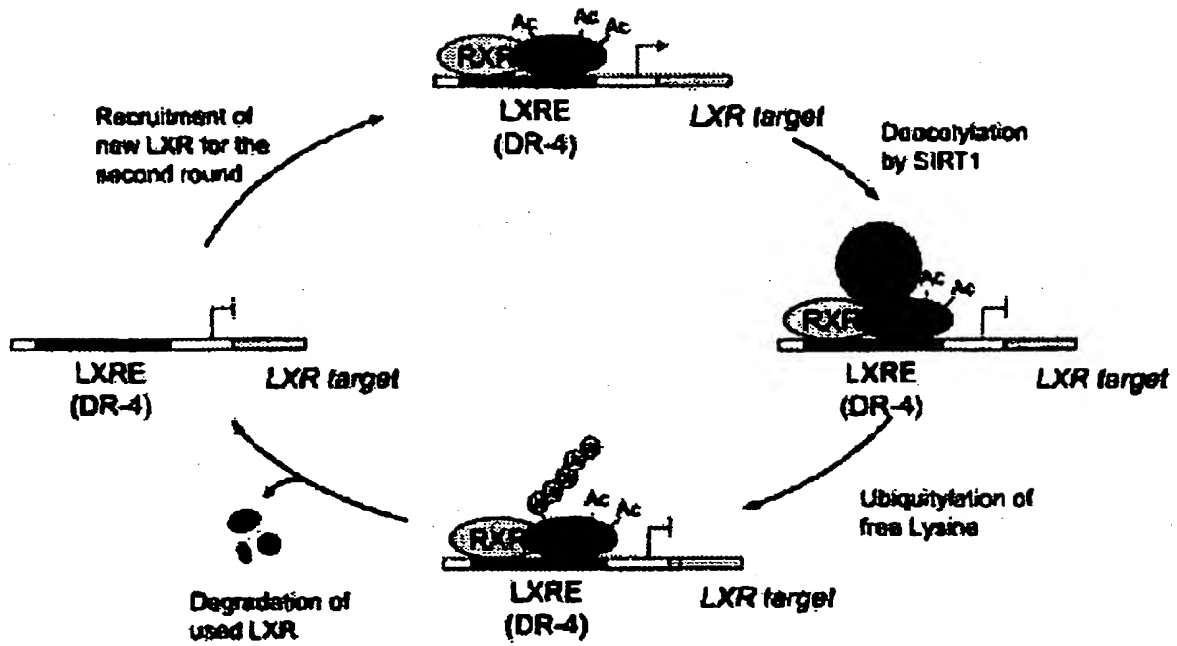


Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 07/13364

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68, G01N 33/53 (2007.01)

USPC - 436/500; 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC- 436/500; 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 436/514 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (USPT, PGPB, EPAB, JPAB) and Google Patent/Scholar
Search terms: SIRT1, LXR, cholesterol, deacetylase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,835,563 B1 (Lawn et al.) 28 December 2004 (28.12.2004) col 5, ln 13-18, col 8, ln 47-67, col 9, ln 19-50, col 10, ln 4-32, col 72, ln 27-44 and col 74, ln 12-16,	21
Y		1-20, 22-23
Y	US 2005/0136429 A1 (Guarente et al.) 23 June 2005 (23.06.2005) para [0006], [0010], [0011], [0027], [0028], [0058], [0065], [0066], [0163], [0164], [0196], [0332], [0349] and [0354]	1-20, 22-23
Y	US 2003/0228607 A1 (Wagner et al.) 11 December 2003 (11.12.2003) para [0163]	9, 18

☐ Further documents are listed in the continuation of Box C.

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"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

28 September 2007 (28.09.2007)

Date of mailing of the international search report

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Name and mailing address of the ISA/US

Mall Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774